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(54) Title: NUCLEOTIDE AND PROTEIN SEQUENCES OF VERTEBRATE DELTA GENES AND METHODS BASED THEREON			
(57) Abstract <p>The present invention relates to nucleotide sequences of vertebrate <i>Delta</i> genes, and amino acid sequences of their encoded proteins, as well as derivatives (e.g., fragments) and analogs thereof. In a specific embodiment, the vertebrate Delta protein is a human protein. The invention further relates to fragments (and derivatives and analogs thereof) of Delta which comprise one or more domains of the Delta protein, including but not limited to the intracellular domain, extracellular domain, DSL domain, domain amino-terminal to the DSL domain, transmembrane region, or one or more EGF-like repeats of a Delta protein, or any combination of the foregoing. Antibodies to Delta, its derivatives and analogs, are additionally provided. Methods of production of the Delta proteins, derivatives and analogs, e.g., by recombinant means, are also provided. Therapeutic and diagnostic methods and pharmaceutical compositions are provided. In specific examples, isolated Delta genes, from <i>Xenopus</i>, chick, mouse, and human, are provided.</p>			

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NUCLEOTIDE AND PROTEIN SEQUENCES OF
VERTEBRATE DELTA GENES AND METHODS BASED THEREON

This application claims priority to United States
5 Provisional Application Serial No. 60/000,589 filed June 28,
1995, which is incorporated by reference herein in its
entirety.

1. INTRODUCTION

10 The present invention relates to vertebrate *Delta*
genes and their encoded protein products, as well as
derivatives and analogs thereof. Production of vertebrate
Delta proteins, derivatives, and antibodies is also provided.
The invention further relates to therapeutic compositions and
15 methods of diagnosis and therapy.

2. BACKGROUND OF THE INVENTION

Genetic analyses in *Drosophila* have been extremely
useful in dissecting the complexity of developmental pathways
20 and identifying interacting loci. However, understanding the
precise nature of the processes that underlie genetic
interactions requires a knowledge of the protein products of
the genes in question.

The vertebrate central nervous system is an
25 intimate mixture of different cell types, almost all
generated from the same source - the neurogenic epithelium
that forms the neural plate and subsequently the neural tube.
What are the mechanisms that control neurogenesis in this
sheet of cells, directing some to become neurons while others
30 remain non-neuronal? The answer is virtually unknown for
vertebrates, but many of the cellular interactions and genes
controlling cell fate decisions during neurogenesis have been
well characterized in *Drosophila* (Campos-Ortega, 1993, J.
Neurobiol. 24:1305-1327). Although the gross anatomical
35 context of neurogenesis appears very different in insects and
vertebrates, the possibility remains that, at a cellular
level, similar events are occurring via conserved molecular

mechanisms. Embryological, genetic and molecular evidence indicates that the early steps of ectodermal differentiation in *Drosophila* depend on cell interactions (Doe and Goodman, 1985, Dev. Biol. 111:206-219; Technau and Campos-Ortega, 1986, Dev. Biol. 195:445-454; Vässin et al., 1985, J. Neurogenet. 2:291-308; de la Concha et al., 1988, Genetics 118:499-508; Xu et al., 1990, Genes Dev. 4:464-475; Artavanis-Tsakonas, 1988, Trends Genet. 4:95-100).

Mutational analyses reveal a small group of zygotically-acting genes, the so called neurogenic loci, which affect the choice of ectodermal cells between epidermal and neural pathways (Poulson, 1937, Proc. Natl. Acad. Sci. 23:133-137; Lehmann et al., 1983, Wilhelm Roux's Arch. Dev. Biol. 192:62-74; Jürgens et al., 1984, Wilhelm Roux's Arch. Dev. Biol. 193:283-295; Wieschaus et al., 1984, Wilhelm Roux's Arch. Dev. Biol. 193:296-307; Nüsslein-Volhard et al., 1984, Wilhelm Roux's Arch. Dev. Biol. 193:267-282). Null mutations in any one of the zygotic neurogenic loci -- *Notch* (*N*), *Delta* (*Dl*), *mastermind* (*mam*), *Enhancer of Split* (*E(spl)*), *neuralized* (*neu*), and *big brain* (*bib*) -- result in hypertrophy of the nervous system at the expense of ventral and lateral epidermal structures. This effect is due to the misrouting of epidermal precursor cells into a neuronal pathway, and implies that neurogenic gene function is necessary to divert cells within the neurogenic region from a neuronal fate to an epithelial fate.

Neural precursors arise in the *Drosophila* embryo from a neurogenic epithelium during successive waves of neurogenesis (Campos-Ortega & Hartenstein, 1985, The embryonic development of *Drosophila melanogaster* (Springer-Verlag, Berlin; New York); Doe, 1992, Development 116:855-863). The pattern of production of these cells is largely determined by the activity of the proneural and neurogenic genes. Proneural genes predispose clusters of cells to a neural fate (reviewed in Skeath & Carroll, 1994, Faseb J. 8:714-21), but only a subset of cells in a cluster become neural precursors. This restriction is due to the

- action of the neurogenic genes, which mediate lateral inhibition - a type of inhibitory cell signaling by which a cell committed to a neural fate forces its neighbors either to remain uncommitted or to enter a non-neural pathway
- 5 (Artavanis-Tsakonas & Simpson, 1991, Trends Genet. 7:403-408; Doe & Goodman, 1985, Dev. Biol. 111:206-219). Mutations leading to a failure of lateral inhibition cause an overproduction of neurons - the "neurogenic" phenotype (Lehmann et al., 1981, Roux's Arch. Dev. Biol. 190:226-229;
- 10 Lehmann et al., Roux's Arch. Dev. Biol. 192:62-74). In *Drosophila*, the inhibitory signal is delivered by a transmembrane protein encoded by the *Delta* neurogenic gene, which is displayed by the nascent neural cells (Heitzler & Simpson, 1991, Cell 64:1083-1092). Neighboring cells express
- 15 a transmembrane receptor protein, encoded by the neurogenic gene *Notch* (Fortini & Artavanis-Tsakonas, 1993, Cell 75:1245-1247). *Delta* has been identified as a genetic unit capable of interacting with the *Notch* locus (Xu et al., 1990, Genes Dev. 4:464-475).
- 20 Mutational analyses also reveal that the action of the neurogenic genes is pleiotropic and is not limited solely to embryogenesis. For example, ommatidial, bristle and wing formation, which are known also to depend upon cell interactions, are affected by neurogenic mutations (Morgan et
- 25 al., 1925, Bibliogr. Genet. 2:1-226; Welshons, 1956, Dros. Inf. Serv. 30:157-158; Preiss et al., 1988, EMBO J. 7:3917-3927; Shellenbarger and Mohler, 1978, Dev. Biol. 62:432-446; Technau and Campos-Ortega, 1986, Wilhelm Roux's Dev. Biol. 195:445-454; Tomlison and Ready, 1987, Dev. Biol. 120:366-
- 30 376; Cagan and Ready, 1989, Genes Dev. 3:1099-1112). Neurogenic genes are also required for normal development of the muscles, gut, excretory and reproductive systems of the fly (Muskavitch, 1994, Dev. Biol. 166:415-430).
- Both *Notch* and *Delta* are transmembrane proteins
- 35 that span the membrane a single time (Wharton et al., 1985, Cell 43:567-581; Kidd and Young, 1986, Mol. Cell. Biol. 6:3094-3108; Vässin, et al., 1987, EMBO J. 6:3431-3440;

Kopczynski, et al., 1988, *Genes Dev.* 2:1723-1735) and include multiple tandem EGF-like repeats in their extracellular domains (Muskavitch, 1994, *Dev. Biol.* 166:415-430). The *Notch* gene encodes a ~300 kd protein (we use "Notch" to denote this protein) with a large N-terminal extracellular domain that includes 36 epidermal growth factor (EGF)-like tandem repeats followed by three other cysteine-rich repeats, designated *Notch/lin-12* repeats (Wharton, et al., 1985, *Cell* 43:567-581; Kidd and Young, 1986, *Mol. Cell. Biol.* 6:3094-3108; Yochem, et al., 1988, *Nature* 335:547-550). Molecular studies have lead to the suggestion that Notch and Delta constitute biochemically interacting elements of a cell communication mechanism involved in early developmental decisions (Fehon et al., 1990, *Cell* 61:523-534). Homologs are found in *Caenorhabditis elegans*, where the *Notch*-related gene *lin-12* and the *Delta*-related gene *lag-2* are also responsible for lateral inhibition (Sternberg, 1993, *Current Biol.* 3:763-765; Henderson et al., 1994, *Development* 120:2913-2924; Greenwald, 1994, *Curr. Opin. Genet. Dev.* 4:556-562). In vertebrates, several *Notch* homologs have also been identified (Kopan & Weintraub, 1993, *J. Cell Biol.* 121:631-641; Lardelli et al., 1994, *Mech. Dev.* 46:123-136; Lardelli & Lendahl, 1993, *Exp. Cell Res.* 204:364-372; Weinmaster et al., 1991, *Development* 113:199-205; Weinmaster et al., 1992, *Development* 116:931-941; Coffman et al., 1990, *Science* 249:1438-1441; Bierkamp & Campos-Ortega, 1993, *Mech. Dev.* 43:87-100), and they are expressed in many tissues and at many stages of development. Loss of *Notch-1* leads to somite defects and embryonic death in mice (Swiatek et al., 1994, *Genes Dev.* 8:707-719; Conlon et al., Rossant, J. *Development* (J. *Dev.* 121:1533-1545), while constitutively active mutant forms of *Notch-1* appear to inhibit cell differentiation in *Xenopus* and in cultured mammalian cells (Coffman et al., 1993, *Cell* 73:659-671; Kopan et al., 1994, *Development* 120:2385-2396; Nye et al., 1994, *Development* 120:2421-2430).

The EGF-like motif has been found in a variety of proteins, including those involved in the blood clotting cascade (Furie and Furie, 1988, Cell 53: 505-518). In particular, this motif has been found in extracellular
5 proteins such as the blood clotting factors IX and X (Rees et al., 1988, EMBO J. 7:2053-2061; Furie and Furie, 1988, Cell 53: 505-518), in other *Drosophila* genes (Knust et al., 1987 EMBO J. 761-766; Rothberg et al., 1988, Cell 55:1047-1059), and in some cell-surface receptor proteins, such as
10 thrombomodulin (Suzuki et al., 1987, EMBO J. 6:1891-1897) and LDL receptor (Sudhof et al., 1985, Science 228:815-822). A protein binding site has been mapped to the EGF repeat domain in thrombomodulin and urokinase (Kurosawa et al., 1988, J. Biol. Chem 263:5993-5996; Appella et al., 1987, J. Biol.
15 Chem. 262:4437-4440).

Citation of references hereinabove shall not be construed as an admission that such references are prior art to the present invention.

20

3. SUMMARY OF THE INVENTION

The present invention relates to nucleotide sequences of vertebrate *Delta* genes (chick and mouse *Delta*, and related genes of other species), and amino acid sequences of their encoded proteins, as well as derivatives (e.g.,
25 fragments) and analogs thereof. Nucleic acids hybridizable to or complementary to the foregoing nucleotide sequences are also provided. In a specific embodiment, the *Delta* protein is a mammalian protein, preferably a human protein.

The invention relates to vertebrate *Delta*
30 derivatives and analogs of the invention which are functionally active, i.e., they are capable of displaying one or more known functional activities associated with a full-length (wild-type) *Delta* protein. Such functional activities include but are not limited to antigenicity [ability to bind
35 (or compete with *Delta* for binding) to an anti-*Delta* antibody], immunogenicity (ability to generate antibody which binds to *Delta*), ability to bind (or compete with *Delta* for

binding) to Notch or other toporythmic proteins or fragments thereof ("adhesiveness"), ability to bind (or compete with Delta for binding) to a receptor for Delta. "Toporythmic proteins" as used herein, refers to the protein products of
5 *Notch*, *Delta*, *Serrate*, *Enhancer of split*, and *Deltex*, as well as other members of this interacting set of genes which may be identified, e.g., by virtue of the ability of their gene sequences to hybridize, or their homology to *Delta*, *Serrate*, or *Notch*, or the ability of their genes to display phenotypic
10 interactions or the ability of their protein products to interact biochemically.

The invention further relates to fragments (and derivatives and analogs thereof) of a vertebrate *Delta* that comprise one or more domains of the *Delta* protein, including
15 but not limited to the intracellular domain, extracellular domain, transmembrane domain, DSL domain, domain amino-terminal to the DSL domain, or one or more EGF-like (homologous) repeats of a *Delta* protein, or any combination of the foregoing.

20 Antibodies to a vertebrate *Delta*, its derivatives and analogs, are additionally provided.

Methods of production of the vertebrate *Delta* proteins, derivatives and analogs, e.g., by recombinant means, are also provided.

25 The present invention also relates to therapeutic and diagnostic methods and compositions based on *Delta* proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention.

30 Such therapeutic compounds (termed herein "Therapeutics") include: *Delta* proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the *Delta* proteins, analogs, or derivatives; and *Delta* antisense nucleic acids. In a preferred

35 embodiment, a Therapeutic of the invention is administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a

malignant state. In other specific embodiments, a Therapeutic of the invention is administered to treat a nervous system disorder or to promote tissue regeneration and repair.

- 5 In one embodiment, Therapeutics which antagonize, or inhibit, Notch and/or Delta function (hereinafter "Antagonist Therapeutics") are administered for therapeutic effect. In another embodiment, Therapeutics which promote Notch and/or Delta function (hereinafter "Agonist
10 Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (e.g., cancer) or hypoproliferative disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch and/or Delta
15 protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of Delta which mediates
20 binding to a Notch protein or a fragment thereof.

3.1. DEFINITIONS

As used herein, underscoring or italicizing the name of a gene shall indicate the gene, in contrast to its
25 encoded protein product which is indicated by the name of the gene in the absence of any underscoring. For example, "Delta" shall mean the Delta gene, whereas "Delta" shall indicate the protein product of the Delta gene.

30 4. DESCRIPTION OF THE FIGURES

Figure 1A-1B. 1A. The DNA sequence of chick Delta (C-Delta-1) (SEQ ID NO:1). 1B. The DNA sequence of an alternatively spliced chick Delta (C-Delta-1) (SEQ ID NO:3).

Figure 2. The predicted amino acid sequence of chick
35 Delta (C-Delta-1) (SEQ ID NO:2).

Figure 3. Predicted amino acid sequence of C-Delta-1 (SEQ ID NO:2), aligned with that of X-Delta-1 (*Xenopus* Delta;

SEQ ID NO:5) and *Drosophila* Delta (SEQ ID NO:6) and, indicating the conserved domain structures: EGF repeats, DSL domain, and transmembrane domain (TM). Conserved amino acids are boxed, and ● denote aligned and non-aligned N-terminal cysteine residues, respectively. Although the intracellular domains of C-Delta-1 and X-Delta-1 closely resemble each other, they show no significant homology to the corresponding part of *Drosophila* Delta.

Figure 4. Alignment of DSL domains from C-Delta-1 (SEQ ID NO:2), *Drosophila* Delta (SEQ ID NO:6) (Vässin et al., 1987, EMBO J. 6:3431-3440; Kopczynski et al., 1988, Genes Dev. 2:1723-1735), *Drosophila* Serrate (SEQ ID NO:7) (Fleming et al., 1990, Genes Dev. 4:2188-2201; Thomas et al., 1991, Development 111:749-761), C-Serrate-1 (SEQ ID NO:8) (Myat, Henrique, Ish-Horowicz and Lewis, in preparation), Apx-1 (SEQ ID NO:9) (Mello et al., 1994, Cell 77:95-106) and Lag-2 (SEQ ID NO:10) (Henderson et al., 1994, Development 120:2913-2924; Tax et al., 1994, Nature 368:150-154), showing the conserved Cysteine spacings, the amino acids that are conserved between presumed ligands for Notch-like proteins in *Drosophila* and vertebrates, and those that are further conserved in *C. elegans* ligands (boxes).

Figure 5A-5E. *C-Delta-1* and *C-Notch-1* expression correlate with onset of neurogenesis in the one-day (E1) neural plate. Anterior is to the left. Wholemount *in situ* hybridization specimens are shown in Figure 5a-d; 5e is a section. Figure 5a, At stage 7, *C-Notch-1* is expressed throughout most of the neural plate and part of the underlying presomitic mesoderm. Figure 5b, *C-Delta-1* at stage 7 is already detectable in the neural plate, in the future posterior hindbrain, just anterior to the first somite (white box). The posterior end of this neural domain is roughly level with the anterior margin of a domain of very strong expression in the underlying presomitic mesoderm (psm). Earlier expression in the neural plate may occur and be masked by expression in the underlying mesoderm (unpublished results). Figure 5c, Higher magnification view

of the area boxed in 5b, showing scattered cells in the neural plate expressing *C-Delta-1*. Figure 5d, At stage 8, *C-Delta-1* expression in the neural plate extends posteriorly as the neural plate develops. The domain of labelled neural plate cells visible in this photograph (bracketed) continues posteriorly over the presomitic mesoderm. Figure 5e, Parasagittal section of a stage 8 embryo showing that *C-Delta-1* is expressed in scattered cells of the neural plate (dorsal layer of tissue; bracketed), and broadly in the presomitic mesoderm (ventral layer). The plane of section is slightly oblique, missing the posterior part of the neural plate domain (cf. 5d).

Figure 6A-6C. *C-Delta-1*-expressing cells do not incorporate BrdU. Of 612 *C-Delta-1* cells, 581 were BrdU⁻ (76 sections; 6 embryos). Figure 6a, Diagram showing how phase in the cell cycle is related to apico-basal position of the nucleus for cells in the neuroepithelium; S-phase nuclei lie basally (Fujita, 1963, J. Comp. Neurol. 120:37-42; Biffo et al., 1992, Histochem. Cytochem. 40:535-540). Nuclei are indicated by shading. Figure 6b, Section through the neural tube of a stage 9 embryo labelled for 2 h with BrdU showing *C-Delta-1* expressing cells (dark on blue background) and BrdU-labelled nuclei (pink). Labelled nuclei are predominantly basal, where DNA synthesis occurs, yet basal *C-Delta-1*-expressing cells are unlabelled. Figure 6c, Section through a stage 9 embryo incubated for 4h: many labelled nuclei have exited S-phase and have moved towards the lumen, but *C-Delta-1*-expressing cells are still basal and not labelled with BrdU.

Figure 7. The DNA sequence of mouse *Delta* (M-*Delta-1*) (SEQ ID NO:11).

Figure 8. The predicted amino acid sequence of the mouse *Delta* (M-*Delta-1*) (SEQ ID NO:12).

Figure 9. An alignment of the predicted amino acid sequence of mouse M-*Delta-1* (SEQ ID NO:12) with the chick *C-Delta-1* (SEQ ID NO:2) which shows their extensive amino acid sequence identity. Identical amino acids are boxed. The

consensus sequence between the two genes is at the bottom (SEQ ID NO:13).

Figure 10. The DNA sequence of a PCR amplified fragment of human *Delta* (H-Delta-1) (SEQ ID NO:14) and the predicted amino acid sequences using the three available open reading frames, 2nd line (SEQ ID NO:15), 3rd line (SEQ ID NO:16), 4th line (SEQ ID NO:17).

Figure 11. An alignment of human H-Delta-1 (top line) and chick C-Delta-1 (bottom line). The predicted amino acid sequence of human *Delta* (SEQ ID NO:18) is shown in the top line. The sequence of human *Delta* was determined by "eye", in which the sequence of the appropriate reading frame was determined by maximizing homology with C-Delta-1. No single reading frame shown in Figure 10 gave the correct sequence due to errors in the DNA sequence of Figure 10 that caused reading frameshifts.

Figure 12A-12B. Figure 12A presents the contig DNA sequence of human *Delta* (H-Delta-1) (SEQ ID NO:33) from clone HD1 18. Figure 12B presents the nucleotide sequence shown in Figure 12A (top line, SEQ ID NO:33) and the deduced amino acid sequences using the three possible open reading frames, second line (SEQ ID NO:34), third line (SEQ ID NO:35), fourth line (SEQ ID NO:36). The amino acid sequence with the greatest homology to the mouse *Delta*-1 amino acid sequence is boxed. This boxed amino acid sequence is the predicted amino acid sequence of human *Delta*; where the reading frame shifts indicates where a sequencing error is present in the sequence. No single reading frame shown in Figure 12A gave an uninterrupted amino acid sequence due to errors in the DNA sequence that caused shifts in the reading frame. X indicates an undetermined amino acid; N indicates an undetermined nucleotide.

Figure 13. An alignment of mouse M-Delta-1 DNA sequence (top line, SEQ ID NO:37) and human H-Delta-1 DNA sequence (second line, SEQ ID NO:33) and their consensus sequence (third line, SEQ ID NO:38).

Figure 14. The composite human Delta (H-Delta-1) amino acid sequence (SEQ ID NOS:39-65, respectively) is presented, representing the boxed amino sequence from Figure 12B. ">" indicates that the sequence continues on the line below. "*" indicates a break in the sequence.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to nucleotide sequences of vertebrate *Delta* genes, and amino acid sequences of their encoded proteins. The invention further relates to fragments and other derivatives, and analogs, of vertebrate *Delta* proteins. Nucleic acids encoding such fragments or derivatives are also within the scope of the invention. The invention provides *Delta* genes and their encoded proteins of many different vertebrate species. The *Delta* genes of the invention include chick, mouse, and human *Delta* and related genes (homologs) in other vertebrate species. In specific embodiments, the *Delta* genes and proteins are from vertebrates, or more particularly, mammals. In a preferred embodiment of the invention, the *Delta* protein is a human protein. Production of the foregoing proteins and derivatives, e.g., by recombinant methods, is provided.

The invention relates to *Delta* derivatives and analogs of the invention which are functionally active, i.e., they are capable of displaying one or more known functional activities associated with a full-length (wild-type) *Delta* protein. Such functional activities include but are not limited to antigenicity [ability to bind (or compete with *Delta* for binding) to an anti-*Delta* antibody], immunogenicity (ability to generate antibody which binds to *Delta*), ability to bind (or compete with *Delta* for binding) to Notch or other toporythmic proteins or fragments thereof ("adhesiveness"), ability to bind (or compete with *Delta* for binding) to a receptor for *Delta*, ability to affect cell fate differentiation, and therapeutic activity. "Toporythmic proteins" as used herein, refers to the protein products of *Notch*, *Delta*, *Serrate*, *Enhancer of split*, and *Deltex*, as well

as other members of this interacting gene family which may be identified, e.g., by virtue of the ability of their gene sequences to hybridize, or their homology to Delta, Serrate, or Notch, or the ability of their genes to display phenotypic interactions.

The invention further relates to fragments (and derivatives and analogs thereof) of Delta which comprise one or more domains of the Delta protein, including but not limited to the intracellular domain, extracellular domain, DSL domain, region amino-terminal to the DSL domain, transmembrane domain, membrane-associated region, or one or more EGF-like (homologous) repeats of a Delta protein, or any combination of the foregoing.

Antibodies to vertebrate Delta, its derivatives and analogs, are additionally provided.

As demonstrated *infra*, Delta plays a critical role in development and other physiological processes, in particular, as a ligand to Notch, which is involved in cell fate (differentiation) determination. In particular, Delta is believed to play a major role in determining cell fates in the central nervous system. The nucleic acid and amino acid sequences and antibodies thereto of the invention can be used for the detection and quantitation of Delta mRNA and protein of human and other species, to study expression thereof, to produce Delta and fragments and other derivatives and analogs thereof, in the study and manipulation of differentiation and other physiological processes. The present invention also relates to therapeutic and diagnostic methods and compositions based on Delta proteins and nucleic acids. The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Delta proteins and analogs and derivatives (including fragments) thereof; antibodies thereto; nucleic acids encoding the Delta proteins, analogs, or derivatives; and Delta antisense nucleic acids. In a preferred embodiment, a Therapeutic of the invention is

administered to treat a cancerous condition, or to prevent progression from a pre-neoplastic or non-malignant state into a neoplastic or a malignant state. In other specific embodiments, a Therapeutic of the invention is administered
5 to treat a nervous system disorder or to promote tissue regeneration and repair.

In one embodiment, Therapeutics which antagonize, or inhibit, Notch and/or Delta function (hereinafter "Antagonist Therapeutics") are administered for therapeutic
10 effect. In another embodiment, Therapeutics which promote Notch and/or Delta function (hereinafter "Agonist Therapeutics") are administered for therapeutic effect.

Disorders of cell fate, in particular hyperproliferative (e.g., cancer) or hypoproliferative
15 disorders, involving aberrant or undesirable levels of expression or activity or localization of Notch and/or Delta protein can be diagnosed by detecting such levels, as described more fully *infra*.

In a preferred aspect, a Therapeutic of the
20 invention is a protein consisting of at least a fragment (termed herein "adhesive fragment") of Delta which mediates binding to a Notch protein or a fragment thereof.

The invention is illustrated by way of examples *infra* which disclose, *inter alia*, the cloning of a chick
25 Delta homolog (Section 6), the cloning of a mouse Delta homolog (Section 7), and the cloning of a human Delta homolog (Section 8).

For clarity of disclosure, and not by way of limitation, the detailed description of the invention is
30 divided into the subsections which follow.

5.1. ISOLATION OF THE DELTA GENES

The invention relates to the nucleotide sequences of vertebrate Delta nucleic acids. In specific embodiments,
35 human Delta nucleic acids comprise the cDNA sequences shown in Figure 10 (SEQ ID NO:14) or in Figure 12A (SEQ ID NO:33), or the coding regions thereof, or nucleic

acids encoding a vertebrate Delta protein (e.g., having the sequence of SEQ ID NO:1, 3, 11, 14 or 33). The invention provides nucleic acids consisting of at least 8 nucleotides (i.e., a hybridizable portion) of a vertebrate Delta

5 sequence; in other embodiments, the nucleic acids consist of at least 25 (continuous) nucleotides, 50 nucleotides, 100 nucleotides, 150 nucleotides, or 200 nucleotides of a Delta sequence, or a full-length Delta coding sequence. The invention also relates to nucleic acids hybridizable to or

10 complementary to the foregoing sequences or their complements. In specific aspects, nucleic acids are provided which comprise a sequence complementary to at least 10, 25, 50, 100, or 200 nucleotides or the entire coding region of a vertebrate Delta gene. In a specific embodiment, a nucleic

15 acid which is hybridizable to a vertebrate (e.g., mammalian) Delta nucleic acid (e.g., having sequence SEQ ID NO:14 or SEQ ID NO:33, or an at least 10, 25, 50, 100, or 200 nucleotide portion thereof), or to a nucleic acid encoding a Delta derivative, under conditions of low stringency is provided.

20 By way of example and not limitation, procedures using such conditions of low stringency are as follows (see also Shilo and Weinberg, 1981, Proc. Natl. Acad. Sci. USA 78:6789-6792): Filters containing DNA are pretreated for 6 h at 40°C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl

25 (pH 7.5), 5 mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 µg/ml denatured salmon sperm DNA. Hybridizations are carried out in the same solution with the following modifications: 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 µg/ml salmon sperm DNA, 10% (wt/vol) dextran sulfate, and 5-20 X 10⁶ cpm

30 ³²P-labeled probe is used. Filters are incubated in hybridization mixture for 18-20 h at 40°C, and then washed for 1.5 h at 55°C in a solution containing 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS. The wash solution is replaced with fresh solution and incubated an

35 additional 1.5 h at 60°C. Filters are blotted dry and exposed for autoradiography. If necessary, filters are washed for a third time at 65-68°C and reexposed to film.

other conditions of low stringency which may be used are well known in the art (e.g., as employed for cross-species hybridizations).

In another specific embodiment, a nucleic acid which is hybridizable to a vertebrate (e.g., mammalian) Delta nucleic acid under conditions of high stringency is provided. By way of example and not limitation, procedures using such conditions of high stringency are as follows:

Prehybridization of filters containing DNA is carried out for 10-8 h to overnight at 65°C in buffer composed of 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 µg/ml denatured salmon sperm DNA. Filters are hybridized for 48 h at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 h in a solution containing 2X SSC, 0.01% PVP, 0.01% Ficoll, and 0.01% BSA. This is followed by a wash in 0.1X SSC at 50°C for 45 min before autoradiography. Other conditions of high stringency which may be used are well known in the art.

Nucleic acids encoding fragments and derivatives of vertebrate Delta proteins (see Section 5.6), and Delta antisense nucleic acids (see Section 5.11) are additionally provided. As is readily apparent, as used herein, a "nucleic acid encoding a fragment or portion of a Delta protein" shall be construed as referring to a nucleic acid encoding only the recited fragment or portion of the Delta protein and not the other contiguous portions of the Delta protein as a continuous sequence.

Fragments of vertebrate Delta nucleic acids comprising regions of homology to other toporythmic proteins are also provided. The DSL regions (regions of homology with *Drosophila Serrate* and Delta) of Delta proteins of other species are also provided. Nucleic acids encoding conserved regions between Delta and Serrate, such as those shown in Figures 3 and 8 are also provided.

Specific embodiments for the cloning of a vertebrate *Delta* gene, presented as a particular example but not by way of limitation, follows:

For expression cloning (a technique commonly known
5 in the art), an expression library is constructed by methods known in the art. For example, mRNA (e.g., human) is isolated, cDNA is made and ligated into an expression vector (e.g., a bacteriophage derivative) such that it is capable of being expressed by the host cell into which it is then
10 introduced. Various screening assays can then be used to select for the expressed *Delta* product. In one embodiment, anti-*Delta* antibodies can be used for selection.

In another preferred aspect, PCR is used to amplify the desired sequence in a genomic or cDNA library, prior to
15 selection. Oligonucleotide primers representing known *Delta* sequences (preferably vertebrate sequences) can be used as primers in PCR. In a preferred aspect, the oligonucleotide primers represent at least part of the *Delta* conserved segments of strong homology between *Serrate* and *Delta*. The
20 synthetic oligonucleotides may be utilized as primers to amplify by PCR sequences from a source (RNA or DNA), preferably a cDNA library, of potential interest. PCR can be carried out, e.g., by use of a Perkin-Elmer Cetus thermal cycler and Taq polymerase (Gene Amp[®]). The DNA being
25 amplified can include mRNA or cDNA or genomic DNA from any eukaryotic species. One can choose to synthesize several different degenerate primers, for use in the PCR reactions. It is also possible to vary the stringency of hybridization conditions used in priming the PCR reactions, to allow for
30 greater or lesser degrees of nucleotide sequence similarity between the known *Delta* nucleotide sequence and the nucleic acid homolog being isolated. For cross species hybridization, low stringency conditions are preferred. For same species hybridization, moderately stringent conditions
35 are preferred. After successful amplification of a segment of a *Delta* homolog, that segment may be molecularly cloned and sequenced, and utilized as a probe to isolate a complete

cDNA or genomic clone. This, in turn, will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis, as described *infra*. In this
5 fashion, additional genes encoding Delta proteins may be identified. Such a procedure is presented by way of example in various examples sections *infra*.

The above-methods are not meant to limit the following general description of methods by which clones of
10 Delta may be obtained.

Any vertebrate cell potentially can serve as the nucleic acid source for the molecular cloning of the Delta gene. The nucleic acid sequences encoding Delta can be isolated from mammalian, human, porcine, bovine, feline,
15 avian, equine, canine, as well as additional primate sources, etc. For example, we have amplified fragments of the Delta gene in mouse, chicken, and human, by PCR using cDNA libraries with Delta primers. The DNA may be obtained by standard procedures known in the art from cloned DNA (e.g., a
20 DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell. (See, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York;
25 Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source,
30 the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites
35 using various restriction enzymes. Alternatively, one may use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by

sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

- 5 Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene may be accomplished in a number of ways. For example, if an amount of a portion of a *Delta* (of any species) gene or its specific RNA, or a fragment thereof, 10 e.g., an extracellular domain (see Section 5.6), is available and can be purified and labeled, the generated DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton, W. and Davis, R., 1977, *Science* 196:180; Grunstein, M. And Hogness, D., 1975, *Proc. Natl. Acad. Sci. U.S.A.* 72:3961). Those DNA fragments with substantial 15 homology to the probe will hybridize. It is also possible to identify the appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map if such is 20 available. Further selection can be carried out on the basis of the properties of the gene. Alternatively, the presence of the gene may be detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which 25 hybrid-select the proper mRNAs, can be selected which produce a protein that, e.g., has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, binding activity, *in vitro* aggregation activity ("adhesiveness") or antigenic properties 30 as known for *Delta*. If an antibody to *Delta* is available, the *Delta* protein may be identified by binding of labeled antibody to the putatively *Delta* synthesizing clones, in an ELISA (enzyme-linked immunosorbent assay)-type procedure.

- The *Delta* gene can also be identified by mRNA 35 selection by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA

fragments may represent available, purified Delta DNA of another species (e.g., *Drosophila*). Immunoprecipitation analysis or functional assays (e.g., aggregation ability in vitro; binding to receptor; see *infra*) of the in vitro translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies of Delta cDNA can be synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabelled mRNA or cDNA may then be used as a probe to identify the Delta DNA fragments from among other genomic DNA fragments.

Alternatives to isolating the Delta genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the Delta protein. For example, RNA for cDNA cloning of the Delta gene can be isolated from cells which express Delta. Other methods are possible and within the scope of the invention.

The identified and isolated gene can then be inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may

comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and *Delta* gene may be modified by homopolymeric tailing. Recombinant molecules
5 can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable
10 cloning vector in a "shot gun" approach. Enrichment for the desired gene, for example, by size fractionation, can be done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the
15 isolated *Delta* gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene may be obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the
20 inserted gene from the isolated recombinant DNA.

The *Delta* sequences provided by the instant invention include those nucleotide sequences encoding substantially the same amino acid sequences as found in native vertebrate *Delta* proteins, and those encoded amino
25 acid sequences with functionally equivalent amino acids, all as described in Section 5.6 *infra* for *Delta* derivatives.

5.2. EXPRESSION OF THE *DELTA* GENES

The nucleotide sequence coding for a vertebrate
30 *Delta* protein or a functionally active fragment or other derivative thereof (see Section 5.6), can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary
35 transcriptional and translational signals can also be supplied by the native *Delta* gene and/or its flanking regions. A variety of host-vector systems may be utilized to

express the protein-coding sequence. These include but are not limited to mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, DNA, plasmid DNA, or cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used. In a specific embodiment, the adhesive portion of the *Delta* gene is expressed. In other specific embodiments, the human *Delta* gene is expressed, or a sequence encoding a functionally active portion of human *Delta*. In yet another embodiment, a fragment of *Delta* comprising the extracellular domain, or other derivative, or analog of *Delta* is expressed.

Any of the methods previously described for the insertion of DNA fragments into a vector may be used to construct expression vectors containing a chimeric gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinants (genetic recombination). Expression of nucleic acid sequence encoding a *Delta* protein or peptide fragment may be regulated by a second nucleic acid sequence so that the *Delta* protein or peptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of a *Delta* protein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control *Delta* gene expression include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982,

Nature 296:39-42); prokaryotic expression vectors such as the β -lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-5 25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; plant expression vectors comprising the nopaline synthetase promoter region (Herrera-Estrella et al., Nature 303:209-213) or the cauliflower mosaic virus 35S RNA promoter (Gardner, et al., 10 1981, Nucl. Acids Res. 9:2871), and the promoter of the photosynthetic enzyme ribulose biphosphate carboxylase (Herrera-Estrella et al., 1984, Nature 310:115-120); promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK 15 (phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et 20 al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is 25 active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 30 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58; alpha 1- 35 antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells

(Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-5 2 gene control region which is active in skeletal muscle (Sani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

Expression vectors containing *Delta* gene inserts
10 can be identified by three general approaches: (a) nucleic acid hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid
15 hybridization using probes comprising sequences that are homologous to an inserted toporythmic gene. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase
20 activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. For example, if the *Delta* gene is inserted within the marker gene sequence of the vector, recombinants containing the *Delta*
25 insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign gene product expressed by the recombinant. Such assays can be based, for example, on the physical or functional properties
30 of the *Delta* gene product in vitro assay systems, e.g., aggregation (binding) with Notch, binding to a receptor, binding with antibody.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may
35 be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As

previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (e.g., lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus, expression of the genetically engineered Delta protein may be controlled. Furthermore, different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, cleavage [e.g., of signal sequence]) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of a heterologous mammalian Delta protein. Furthermore, different vector/host expression systems may effect processing reactions such as proteolytic cleavages to different extents.

In other specific embodiments, the Delta protein, fragment, analog, or derivative may be expressed as a fusion, or chimeric protein product (comprising the protein, fragment, analog, or derivative joined via a peptide bond to a heterologous protein sequence (of a different protein)). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a

chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer.

Both cDNA and genomic sequences can be cloned and expressed.

5

5.3. IDENTIFICATION AND PURIFICATION OF THE *DELTA* GENE PRODUCTS

In particular aspects, the invention provides amino acid sequences of a vertebrate Delta, preferably a human Delta, and fragments and derivatives thereof which comprise an antigenic determinant (i.e., can be recognized by an antibody) or which are otherwise functionally active, as well as nucleic acid sequences encoding the foregoing.

"Functionally active" material as used herein refers to that material displaying one or more known functional activities associated with a full-length (wild-type) Delta protein, e.g., binding to Notch or a portion thereof, binding to any other Delta ligand, antigenicity (binding to an anti-Delta antibody), etc.

In specific embodiments, the invention provides fragments of a Delta protein consisting of at least 6 amino acids, 10 amino acids, 25 amino acids, 50 amino acids, or of at least 75 amino acids. Molecules comprising such fragments are also provided. In other embodiments, the proteins comprise or consist essentially of an extracellular domain, DSL domain, epidermal growth factor-like repeat (ELR) domain, one or any combination of ELRs, transmembrane domain, or intracellular (cytoplasmic) domain, or a portion which binds to Notch, or any combination of the foregoing, of a vertebrate Delta protein. Fragments, or proteins comprising fragments, lacking some or all of the foregoing regions of a Delta protein are also provided. Nucleic acids encoding the foregoing are provided.

Once a recombinant which expresses the Delta gene sequence is identified, the gene product can be analyzed. This is achieved by assays based on the physical or functional properties of the product, including radioactive

labelling of the product followed by analysis by gel electrophoresis, immunoassay, etc.

Once the Delta protein is identified, it may be isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties may be evaluated using any suitable assay (see Section 5.7).

Alternatively, once a Delta protein produced by a recombinant is identified, the amino acid sequence of the protein can be deduced from the nucleotide sequence of the chimeric gene contained in the recombinant. As a result, the protein can be synthesized by standard chemical methods known in the art (e.g., see Hunkapiller, M., et al., 1984, Nature 310:105-111).

In a specific embodiment of the present invention, such Delta proteins, whether produced by recombinant DNA techniques or by chemical synthetic methods, include but are not limited to those containing, as a primary amino acid sequence, all or part of the amino acid sequences substantially as depicted in Figures 2, 8, 11 or 14 (SEQ ID NOS:2, 10, 16 and 39-65), as well as fragments and other derivatives, and analogs thereof.

5.4. STRUCTURE OF THE DELTA GENES AND PROTEINS

The structure of the vertebrate Delta genes and proteins can be analyzed by various methods known in the art.

5.4.1. GENETIC ANALYSIS

The cloned DNA or cDNA corresponding to the Delta gene can be analyzed by methods including but not limited to Southern hybridization (Southern, E.M., 1975, J. Mol. Biol. 98:503-517), Northern hybridization (see e.g., Freeman et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:4094-4098), restriction endonuclease mapping (Maniatis, T., 1982, Molecular Cloning, A Laboratory, Cold Spring Harbor, New

York), and DNA sequence analysis. Polymerase chain reaction (PCR; U.S. Patent Nos. 4,683,202, 4,683,195 and 4,889,818; Gyllenstein et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7652-7656; Ochman et al., 1988, Genetics 120:621-623; Loh et al., 1989, Science 243:217-220) followed by Southern hybridization with a Delta-specific probe can allow the detection of the Delta gene in DNA from various cell types. Methods of amplification other than PCR are commonly known and can also be employed. In one embodiment, Southern 10 hybridization can be used to determine the genetic linkage of Delta. Northern hybridization analysis can be used to determine the expression of the Delta gene. Various cell types, at various states of development or activity can be tested for Delta expression. Examples of such techniques and 15 their results are described in Section 6, infra. The stringency of the hybridization conditions for both Southern and Northern hybridization can be manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific Delta probe used.

20 Restriction endonuclease mapping can be used to roughly determine the genetic structure of the Delta gene. Restriction maps derived by restriction analysis. DNA sequence analysis can be performed by any method of Maxam and Gilbert (1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method (Sanger, F., et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699), 25 or use of an automated DNA sequencer (e.g., Applied Biosystems, Foster City, CA).

5.4.2. PROTEIN ANALYSIS

The amino acid sequence of the Delta protein can be 35 derived by deduction from the DNA sequence, or alternatively, by direct sequencing of the protein, e.g., with an automated amino acid sequencer. The amino acid sequence of a

representative Delta protein comprises the sequence substantially as depicted in Figure 2, and detailed in Section 6, *infra*, with the representative mature protein that shown by amino acid numbers 1-728.

5 The Delta protein sequence can be further characterized by a hydrophilicity analysis (Hopp, T. and Woods, K., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824). A hydrophilicity profile can be used to identify the hydrophobic and hydrophilic regions of the Delta protein and
10 the corresponding regions of the gene sequence which encode such regions. Hydrophilic regions are more likely to be immunogenic.

 Secondary, structural analysis (Chou, P. and Fasman, G., 1974, Biochemistry 13:222) can also be done, to
15 identify regions of Delta that assume specific secondary structures.

 Manipulation, translation, and secondary structure prediction, as well as open reading frame prediction and plotting, can also be accomplished using computer software
20 programs available in the art.

 Other methods of structural analysis can also be employed. These include but are not limited to X-ray crystallography (Engstrom, A., 1974, Biochem. Exp. Biol. 11:7-13) and computer modeling (Fletterick, R. and Zoller, M.
25 (eds.), 1986, Computer Graphics and Molecular Modeling, in Current Communications in Molecular Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

5.5. GENERATION OF ANTIBODIES TO DELTA PROTEINS AND DERIVATIVES THEREOF

30 According to the invention, a vertebrate Delta protein, its fragments or other derivatives, or analogs thereof, may be used as an immunogen to generate antibodies which recognize such an immunogen. Such antibodies include
35 but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, antibodies to human Delta are

produced. In another embodiment, antibodies to the extracellular domain of Delta are produced. In another embodiment, antibodies to the intracellular domain of Delta are produced.

5 Various procedures known in the art may be used for the production of polyclonal antibodies to a Delta protein or derivative or analog. In a particular embodiment, rabbit polyclonal antibodies to an epitope of the Delta protein encoded by a sequence depicted in Figures 1a, 1b, 7 or 11, or
10 a subsequence thereof, can be obtained. For the production of antibody, various host animals can be immunized by injection with the native Delta protein, or a synthetic version, or derivative (e.g., fragment) thereof, including but not limited to rabbits, mice, rats, etc. Various
15 adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil
20 emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

For preparation of monoclonal antibodies directed toward a Delta protein sequence or analog thereof, any
25 technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique
30 (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can be
35 produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human

hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus *in vitro* (Cole et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96). In fact, according
5 to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific
10 for Delta together with genes from a human antibody molecule of appropriate biological activity can be used; such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent
15 4,946,778) can be adapted to produce Delta-specific single chain antibodies. An additional embodiment of the invention utilizes the techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal
20 Fab fragments with the desired specificity for Delta proteins, derivatives, or analogs.

Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the
25 F(ab')₂ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing
30 agent.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific
35 domain of a vertebrate Delta protein, one may assay generated hybridomas for a product which binds to a Delta fragment containing such domain. For selection of an antibody

immunospecific to human Delta, one can select on the basis of positive binding to human Delta and a lack of binding to *Drosophila* Delta.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the protein sequences of the invention (e.g., see Section 5.7, *infra*), e.g., for imaging these proteins, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

10 Antibodies specific to a domain of a Delta protein are also provided. In a specific embodiment, antibodies which bind to a Notch-binding fragment of Delta are provided.

In another embodiment of the invention (see *infra*), anti-Delta antibodies and fragments thereof containing the
15 binding domain are Therapeutics.

5.6. DELTA PROTEINS, DERIVATIVES AND ANALOGS

The invention further relates to vertebrate (e.g., mammalian) Delta proteins, and derivatives (including but not
20 limited to fragments) and analogs of vertebrate Delta proteins. Nucleic acids encoding Delta protein derivatives and protein analogs are also provided. In one embodiment, the Delta proteins are encoded by the Delta nucleic acids described in Section 5.1 *supra*. In particular aspects, the
25 proteins, derivatives, or analogs are of mouse, chicken, rat, pig, cow, dog, monkey, or human Delta proteins. In a specific embodiment, a mature, full-length vertebrate Delta protein is provided. In one embodiment, a vertebrate Delta protein lacking only the signal sequence (approximately the
30 first 17 amino-terminal amino acids) is provided.

The production and use of derivatives and analogs related to Delta are within the scope of the present invention. In a specific embodiment, the derivative or analog is functionally active, i.e., capable of exhibiting
35 one or more functional activities associated with a full-length, wild-type Delta protein. As one example, such derivatives or analogs which have the desired immunogenicity

or antigenicity can be used, for example, in immunoassays, for immunization, for inhibition of Delta activity, etc. Such molecules which retain, or alternatively inhibit, a desired Delta property, e.g., binding to Notch or other toporythmic proteins, binding to a cell-surface receptor, can be used as inducers, or inhibitors, respectively, of such property and its physiological correlates. A specific embodiment relates to a Delta fragment that can be bound by an anti-Delta antibody but cannot bind to a Notch protein or other toporythmic protein. Derivatives or analogs of Delta can be tested for the desired activity by procedures known in the art, including but not limited to the assays described in Section 5.7.

In particular, Delta derivatives can be made by altering Delta sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a Delta gene may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of Delta genes which are altered by the substitution of different ccdons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the Delta derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a Delta protein including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine,

isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids
5 include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

In a specific embodiment of the invention, proteins consisting of or comprising a fragment of a vertebrate Delta
10 protein consisting of at least 10 (continuous) amino acids of the Delta protein is provided. In other embodiments, the fragment consists of at least 20 or 50 amino acids of the Delta protein. In specific embodiments, such fragments are not larger than 35, 100 or 200 amino acids. Derivatives or
15 analogs of Delta include but are not limited to those peptides which are substantially homologous to a vertebrate Delta protein or fragments thereof (e.g., at least 30%, 50%, 70%, or 90% identity over an amino acid sequence of identical size -- e.g., comprising a domain) or whose encoding nucleic
20 acid is capable of hybridizing to a coding Delta sequence.

The Delta derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned Delta
25 gene sequence can be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s),
30 followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analog of Delta, care should be taken to ensure that the modified gene remains within the same translational reading frame as Delta, uninterrupted by
35 translational stop signals, in the gene region where the desired Delta activity is encoded.

Additionally, the Delta-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or to create variations in coding regions and/or
5 form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol.
10 Chem 253:6551), use of TAB® linkers (Pharmacia), etc. PCR primers containing sequence changes can be used in PCR to introduce such changes into the amplified fragments.

Manipulations of the Delta sequence may also be made at the protein level. Included within the scope of the
15 invention are Delta protein fragments or other derivatives or analogs which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to
20 an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation,
25 reduction; metabolic synthesis in the presence of tunicamycin; etc.

In addition, analogs and derivatives of Delta can be chemically synthesized. For example, a peptide corresponding to a portion of a Delta protein which comprises
30 the desired domain (see Section 5.6.1), or which mediates the desired aggregation activity *in vitro*, or binding to a receptor, can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or
35 addition into the Delta sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids, α -amino isobutyric acid, 4-aminobutyric acid,

hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, designer amino acids such as β -methyl amino acids, α -methyl amino acids, and $N\alpha$ -methyl amino acids.

In a specific embodiment, the Delta derivative is a chimeric, or fusion, protein comprising a vertebrate Delta protein or fragment thereof (preferably consisting of at least a domain or motif of the Delta protein, or at least 10 amino acids of the Delta protein) joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. In one embodiment, such a chimeric protein is produced by recombinant expression of a nucleic acid encoding the protein (comprising a Delta-coding sequence joined in-frame to a coding sequence for a different protein). Such a chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other by methods known in the art, in the proper coding frame, and expressing the chimeric product by methods commonly known in the art. Alternatively, such a chimeric product may be made by protein synthetic techniques, e.g., by use of a peptide synthesizer. In a specific embodiment, a chimeric nucleic acid encoding a mature Delta protein with a heterologous signal sequence is expressed such that the chimeric protein is expressed and processed by the cell to the mature Delta protein. As another example, and not by way of limitation, a recombinant molecule can be constructed according to the invention, comprising coding portions of both Delta and another toporythmic gene, e.g., Serrate. The encoded protein of such a recombinant molecule could exhibit properties associated with both Serrate and Delta and portray a novel profile of biological activities, including agonists as well as antagonists. The primary sequence of Delta and Serrate may also be used to predict tertiary structure of the molecules using computer simulation (Hopp and Woods, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828); Delta/Serrate chimeric

recombinant genes could be designed in light of correlations between tertiary structure and biological function.

Likewise, chimeric genes comprising portions of Delta fused to any heterologous protein-encoding sequences may be constructed. A specific embodiment relates to a chimeric protein comprising a fragment of Delta of at least six amino acids.

In another specific embodiment, the Delta derivative is a fragment of vertebrate Delta comprising a region of homology with another toporythmic protein. As used herein, a region of a first protein shall be considered "homologous" to a second protein when the amino acid sequence of the region is at least 30% identical or at least 75% either identical or involving conservative changes, when compared to any sequence in the second protein of an equal number of amino acids as the number contained in the region. For example, such a Delta fragment can comprise one or more regions homologous to Serrate, including but not limited to the DSL domain or a portion thereof.

Other specific embodiments of derivatives and analogs are described in the subsections below and examples sections *infra*.

5.6.1. DERIVATIVES OF DELTA CONTAINING ONE OR MORE DOMAINS OF THE PROTEIN

In a specific embodiment, the invention relates to vertebrate Delta derivatives and analogs, in particular Delta fragments and derivatives of such fragments, that comprise, or alternatively consist of, one or more domains of the Delta protein, including but not limited to the extracellular domain, signal sequence, region amino-terminal to the DSL domain, DSL domain, ELR domain, transmembrane domain, intracellular domain, and one or more of the EGF-like repeats (ELR) of the Delta protein (e.g., ELRs 1-9), or any combination of the foregoing. In particular examples relating to the chick and mouse Delta proteins, such domains are identified in Examples Section 6 and 7, respectively, and

in Figures 3 and 9. Thus, by way of example is provided, a molecule comprising an extracellular domain (approximately amino acids 1-545), signal sequence (approximately amino acids 1-17), region amino-terminal to the DSL domain (approximately amino acids 1-178), the DSL domain (approximately amino acids 179-223), EGF1 (approximately amino acids 229-260), EGF2 (approximately amino acids 261-292), EGF3 (approximately amino acids 293-332), EGF4 (approximately amino acids 333-370), EGF5 (approximately amino acids 371-409), EGF6 (approximately amino acids 410-447), EGF7 (approximately amino acids 448-485), EGF8 (approximately amino acids 486-523), transmembrane domain, and intracellular (cytoplasmic) domain (approximately amino acids 555-728) of a vertebrate Delta.

In a specific embodiment, the molecules comprising specific fragments of vertebrate Delta are those comprising fragments in the respective Delta protein most homologous to specific fragments of the *Drosophila* or chick Delta protein. In particular embodiments, such a molecule comprises or consists of the amino acid sequences of SEQ ID NO:2 or 16. Alternatively, a fragment comprising a domain of a Delta homolog can be identified by protein analysis methods as described in Section 5.3.2.

5.6.2. DERIVATIVES OF DELTA THAT MEDIATE BINDING TO TOPORYTHMIC PROTEIN DOMAINS

The invention also provides for vertebrate Delta fragments, and analogs or derivatives of such fragments, which mediate binding to toporythmic proteins (and thus are termed herein "adhesive"), and nucleic acid sequences encoding the foregoing.

In a particular embodiment, the adhesive fragment of a Delta protein comprises the DSL domain, or a portion thereof. Subfragments within the DSL domain that mediate binding to Notch can be identified by analysis of constructs expressing deletion mutants.

The ability to bind to a toporythmic protein (preferably Notch) can be demonstrated by *in vitro* aggregation assays with cells expressing such a toporythmic protein as well as cells expressing Delta or a Delta derivative (See Section 5.7). That is, the ability of a Delta fragment to bind to a Notch protein can be demonstrated by detecting the ability of the Delta fragment, when expressed on the surface of a first cell, to bind to a Notch protein expressed on the surface of a second cell.

10 The nucleic acid sequences encoding toporythmic proteins or adhesive domains thereof, for use in such assays, can be isolated from human, porcine, bovine, feline, avian, equine, canine, or insect, as well as primate sources and any other species in which homologs of known toporythmic genes
15 can be identified.

5.7. ASSAYS OF DELTA PROTEINS, DERIVATIVES AND ANALOGS

The functional activity of vertebrate Delta
20 proteins, derivatives and analogs can be assayed by various methods.

For example, in one embodiment, where one is assaying for the ability to bind or compete with wild-type Delta for binding to anti-Delta antibody, various
25 immunoassays known in the art can be used, including but not limited to competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions,
30 immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays,
35 protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the

primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labelled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where one is assaying for the ability to mediate binding to a toporythmic protein, e.g., Notch, one can carry out an *in vitro* aggregation assay (see Fehon et al., 1990, Cell 61:523-534; Rebay et al., 1991, Cell 67:687-699).

In another embodiment, where a receptor for Delta is identified, receptor binding can be assayed, e.g., by means well-known in the art. In another embodiment, physiological correlates of Delta binding to cells expressing a Delta receptor (signal transduction) can be assayed.

In another embodiment, in insect or other model systems, genetic studies can be done to study the phenotypic effect of a Delta mutant that is a derivative or analog of wild-type Delta.

Other methods will be known to the skilled artisan and are within the scope of the invention.

5.8. THERAPEUTIC USES

The invention provides for treatment of disorders of cell fate or differentiation by administration of a therapeutic compound of the invention. Such therapeutic compounds (termed herein "Therapeutics") include: Delta proteins and analogs and derivatives (including fragments) thereof (e.g., as described hereinabove); antibodies thereto (as described hereinabove); nucleic acids encoding the Delta proteins, analogs, or derivatives (e.g., as described hereinabove); and Delta antisense nucleic acids. As stated *supra*, the Antagonist Therapeutics of the invention are those Therapeutics which antagonize, or inhibit, a Delta function and/or Notch function (since Delta is a Notch ligand). Such Antagonist Therapeutics are most preferably identified by use

of known convenient *in vitro* assays, e.g., based on their ability to inhibit binding of Delta to another protein (e.g., a Notch protein), or inhibit any known Notch or Delta function as preferably assayed *in vitro* or in cell culture, although genetic assays (e.g., in *Drosophila*) may also be employed. In a preferred embodiment, the Antagonist Therapeutic is a protein or derivative thereof comprising a functionally active fragment such as a fragment of Delta which mediates binding to Notch, or an antibody thereto. In other specific embodiments, such an Antagonist Therapeutic is a nucleic acid capable of expressing a molecule comprising a fragment of Delta which binds to Notch, or a Delta antisense nucleic acid (see Section 5.11 herein). It should be noted that preferably, suitable *in vitro* or *in vivo* assays, as described *infra*, should be utilized to determine the effect of a specific Therapeutic and whether its administration is indicated for treatment of the affected tissue, since the developmental history of the tissue may determine whether an Antagonist or Agonist Therapeutic is desired.

In addition, the mode of administration, e.g., whether administered in soluble form or administered via its encoding nucleic acid for intracellular recombinant expression, of the Delta protein or derivative can affect whether it acts as an agonist or antagonist.

In another embodiment of the invention, a nucleic acid containing a portion of a Delta gene is used, as an Antagonist Therapeutic, to promote Delta inactivation by homologous recombination (Koller and Smithies, 1989, Proc. Natl. Acad. Sci. USA 86:8932-8935; Zijlstra et al., 1989, Nature 342:435-438).

The Agonist Therapeutics of the invention, as described *supra*, promote Delta function. Such Agonist Therapeutics include but are not limited to proteins and derivatives comprising the portions of Notch that mediate binding to Delta, and nucleic acids encoding the foregoing (which can be administered to express their encoded products *in vivo*).

Further descriptions and sources of Therapeutics of the inventions are found in Sections 5.1 through 5.7 herein.

Molecules which retain, or alternatively inhibit, a desired Delta property, e.g., binding to Notch, binding to an intracellular ligand, can be used therapeutically as inducers, or inhibitors, respectively, of such property and its physiological correlates. In a specific embodiment, a peptide (e.g., in the range of 6-50 or 15-25 amino acids; and particularly of about 10, 15, 20 or 25 amino acids) containing the sequence of a portion of Delta which binds to Notch is used to antagonize Notch function. In a specific embodiment, such an Antagonist Therapeutic is used to treat or prevent human or other malignancies associated with increased Notch expression (e.g., cervical cancer, colon cancer, breast cancer, squamous adenocarcinomas (see *infra*)). Derivatives or analogs of Delta can be tested for the desired activity by procedures known in the art, including but not limited to the assays described in the examples *infra*. For example, molecules comprising Delta fragments which bind to Notch EGF-repeats (ELR) 11 and 12 and which are smaller than a DSL domain, can be obtained and selected by expressing deletion mutants and assaying for binding of the expressed product to Notch by any of the several methods (e.g., in vitro cell aggregation assays, interaction trap system), some of which are described in the Examples Sections *infra*. In one specific embodiment, peptide libraries can be screened to select a peptide with the desired activity; such screening can be carried out by assaying, e.g., for binding to Notch or a molecule containing the Notch ELR 11 and 12 repeats.

Other Therapeutics include molecules that bind to a vertebrate Delta protein. Thus, the invention also provides a method for identifying such molecules. Such molecules can be identified by a method comprising contacting a plurality of molecules (e.g., in a peptide library, or combinatorial chemical library) with the Delta protein under conditions conducive to binding, and recovering any molecules that bind to the Delta protein.

The Agonist and Antagonist Therapeutics of the invention have therapeutic utility for disorders of cell fate. The Agonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving an absence or decreased (relative to normal, or desired) levels of Notch or Delta function, for example, in patients where Notch or Delta protein is lacking, genetically defective, biologically inactive or underactive, or underexpressed; and (2) in diseases or disorders wherein 10 in vitro (or in vivo) assays (see *infra*) indicate the utility of Delta agonist administration. The absence or decreased levels in Notch or Delta function can be readily detected, e.g., by obtaining a patient tissue sample (e.g., from biopsy tissue) and assaying it in vitro for protein levels, 15 structure and/or activity of the expressed Notch or Delta protein. Many methods standard in the art can be thus employed, including but not limited to immunoassays to detect immunoprecipitation followed by sodium dodecyl sulfate 20 polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect Notch or Delta expression by detecting and/or visualizing respectively Notch hybridization, etc.)

In vitro assays which can be used to determine whether administration of a specific Agonist Therapeutic or Antagonist Therapeutic is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a Therapeutic, and the effect of such Therapeutic upon the tissue sample is observed. In one embodiment, where the patient has a malignancy, a sample of cells from such malignancy is plated out or grown in culture, and the cells are then exposed to a Therapeutic. A Therapeutic which 30 inhibits survival or growth of the malignant cells (e.g., by promoting terminal differentiation) is selected for therapeutic use in vivo. Many assays standard in the art can

be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring ³H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-
5 oncogenes (e.g., *fos*, *myc*) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, etc. In a specific aspect, the malignant cell cultures are separately exposed to (1) an Agonist
10 Therapeutic, and (2) an Antagonist Therapeutic; the result of the assay can indicate which type of Therapeutic has therapeutic efficacy.

In another embodiment, a Therapeutic is indicated for use which exhibits the desired effect, inhibition or
15 promotion of cell growth, upon a patient cell sample from tissue having or suspected of having a hyper- or hypoproliferative disorder, respectively. Such hyper- or hypoproliferative disorders include but are not limited to those described in Sections 5.8.1 through 5.8.3 *infra*.

20 In another specific embodiment, a Therapeutic is indicated for use in treating nerve injury or a nervous system degenerative disorder (see Section 5.8.2) which exhibits *in vitro* promotion of nerve regeneration/neurite extension from nerve cells of the affected patient type.

25 In addition, administration of an Antagonist Therapeutic of the invention is also indicated in diseases or disorders determined or known to involve a Notch or Delta dominant activated phenotype ("gain of function" mutations.) Administration of an Agonist Therapeutic is indicated in
30 diseases or disorders determined or known to involve a Notch or Delta dominant negative phenotype ("loss of function" mutations). The functions of various structural domains of the Notch protein have been investigated *in vivo*, by ectopically expressing a series of *Drosophila* Notch deletion
35 mutants under the hsp70 heat-shock promoter, as well as eye-specific promoters (see Rebay et al., 1993, Cell 74:319-329). Two classes of dominant phenotypes were observed, one

suggestive of *Notch* loss-of function mutations and the other of *Notch* gain-of-function mutations. Dominant "activated" phenotypes resulted from overexpression of a protein lacking most extracellular sequences, while dominant "negative"

5 phenotypes resulted from overexpression of a protein lacking most intracellular sequences. The results indicated that *Notch* functions as a receptor whose extracellular domain mediates ligand-binding, resulting in the transmission of developmental signals by the cytoplasmic domain.

10 In various specific embodiments, *in vitro* assays can be carried out with representative cells of cell types involved in a patient's disorder, to determine if a Therapeutic has a desired effect upon such cell types.

In another embodiment, cells of a patient tissue
15 sample suspected of being pre-neoplastic are similarly plated out or grown *in vitro*, and exposed to a Therapeutic. The Therapeutic which results in a cell phenotype that is more normal (i.e., less representative of a pre-neoplastic state, neoplastic state, malignant state, or transformed phenotype)
20 is selected for therapeutic use. Many assays standard in the art can be used to assess whether a pre-neoplastic state, neoplastic state, or a transformed or malignant phenotype, is present. For example, characteristics associated with a transformed phenotype (a set of *in vitro* characteristics
25 associated with a tumorigenic ability *in vivo*) include a more rounded cell morphology, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, release of proteases such as plasminogen activator, increased sugar transport, decreased serum requirement, expression of fetal
30 antigens, disappearance of the 250,000 dalton surface protein, etc. (see Luria et al., 1978, *General Virology*, 3d Ed., John Wiley & Sons, New York pp. 436-446).

In other specific embodiments, the *in vitro* assays described *supra* can be carried out using a cell line, rather
35 than a cell sample derived from the specific patient to be treated, in which the cell line is derived from or displays characteristic(s) associated with the malignant, neoplastic

or pre-neoplastic disorder desired to be treated or prevented, or is derived from the neural or other cell type upon which an effect is desired, according to the present invention.

5 The Antagonist Therapeutics are administered therapeutically (including prophylactically): (1) in diseases or disorders involving increased (relative to normal, or desired) levels of Notch or Delta function, for example, where the Notch or Delta protein is overexpressed or
10 overactive; and (2) in diseases or disorders wherein *in vitro* (or *in vivo*) assays indicate the utility of Delta antagonist administration. The increased levels of Notch or Delta function can be readily detected by methods such as those described above, by quantifying protein and/or RNA. *In vitro*
15 assays with cells of patient tissue sample or the appropriate cell line or cell type, to determine therapeutic utility, can be carried out as described above.

5.8.1. MALIGNANCIES

20 Malignant and pre-neoplastic conditions which can be tested as described *supra* for efficacy of intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to those described below
25 in Sections 5.8.1 and 5.9.1.

 Malignancies and related disorders, cells of which type can be tested *in vitro* (and/or *in vivo*), and upon observing the appropriate assay result, treated according to the present invention, include but are not limited to those
30 listed in Table 1 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

TABLE 1
MALIGNANCIES AND RELATED DISORDERS

	Leukemia
5	acute leukemia
	acute lymphocytic leukemia
	acute myelocytic leukemia
	myeloblastic
	promyelocytic
	myelomonocytic
	monocytic
	erythroleukemia
10	chronic leukemia
	chronic myelocytic (granulocytic) leukemia
	chronic lymphocytic leukemia
	Polycythemia vera
	Lymphoma
	Hodgkin's disease
	non-Hodgkin's disease
15	Multiple myeloma
	Waldenström's macroglobulinemia
	Heavy chain disease
	Solid tumors
	sarcomas and carcinomas
	fibrosarcoma
	myxosarcoma
20	liposarcoma
	chondrosarcoma
	osteogenic sarcoma
	chordoma
	angiosarcoma
	endotheliosarcoma
	lymphangiosarcoma
	lymphangioendotheliosarcoma
25	synovioma
	mesothelioma
	Ewing's tumor
	leiomyosarcoma
	rhabdomyosarcoma
	colon carcinoma
	pancreatic cancer
30	breast cancer
	ovarian cancer
	prostate cancer
	squamous cell carcinoma
	basal cell carcinoma
	adenocarcinoma
	sweat gland carcinoma
	sebaceous gland carcinoma
35	papillary carcinoma
	papillary adenocarcinomas
	cystadenocarcinoma
	medullary carcinoma

5 bronchogenic carcinoma
 renal cell carcinoma
 hepatoma
 bile duct carcinoma
 choriocarcinoma
 seminoma
 embryonal carcinoma
 Wilms' tumor
 cervical cancer
 testicular tumor
 lung carcinoma
 small cell lung carcinoma
 bladder carcinoma
10 epithelial carcinoma
 glioma
 astrocytoma
 medulloblastoma
 craniopharyngioma
 ependymoma
 pinealoma
 hemangioblastoma
15 acoustic neuroma
 oligodendroglioma
 meningioma
 melanoma
 neuroblastoma
 retinoblastoma

20

 In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias) are treated or prevented in epithelial tissues such as those in the cervix, esophagus, and lung.

25

 Malignancies of the colon and cervix exhibit increased expression of human Notch relative to such non-malignant tissue (see PCT Publication no. WO 94/07474 published April 14, 1994, incorporated by reference herein in its entirety). Thus, in specific embodiments, malignancies

30

or premalignant changes of the colon or cervix are treated or prevented by administering an effective amount of an Antagonist Therapeutic, e.g., a Delta derivative, that antagonizes Notch function. The presence of increased Notch expression in colon, and cervical cancer suggests that many

35

more cancerous and hyperproliferative conditions exhibit upregulated Notch. Thus, in specific embodiments, various

cancers, e.g., breast cancer, squamous adenocarcinoma, seminoma, melanoma, and lung cancer, and premalignant changes therein, as well as other hyperproliferative disorders, can be treated or prevented by administration of an Antagonist
5 Therapeutic that antagonizes Notch function.

5.8.2. NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested as described *supra* for efficacy of
10 intervention with Antagonist or Agonist Therapeutics, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in
15 either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the
20 central (including spinal cord, brain) or peripheral nervous systems:

- (i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;
- 25 (ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;
- 30 (iii) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue;
- 35 (iv) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an

- abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;
- 5 (v) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease,
- 10 Huntington's chorea, or amyotrophic lateral sclerosis;
- (vi) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a
- 15 nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary
- 20 degeneration of the corpus callosum), and alcoholic cerebellar degeneration;
- (vii) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic
- 25 lupus erythematosus, carcinoma, or sarcoidosis;
- (viii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and
- (ix) demyelinated lesions in which a portion of the
- 30 nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies,
- 35 progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons (see also Section 5.8). For example, and not by way of limitation, Therapeutics which elicit any of the following effects may be useful according to the invention:

- (i) increased survival time of neurons in culture;
- (ii) increased sprouting of neurons in culture or
10 *in vivo*;
- (iii) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or
- 15 (iv) decreased symptoms of neuron dysfunction *in vivo*.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et
20 al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic
25 assay, antibody binding, Northern blot assay, etc., depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

30 In a specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well
35 as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to

progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

5.8.3. TISSUE REPAIR AND REGENERATION

In another embodiment of the invention, a Therapeutic of the invention is used for promotion of tissue regeneration and repair, including but not limited to treatment of benign dysproliferative disorders. Specific embodiments are directed to treatment of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes), treatment of keloid (hypertrophic scar) formation (disfiguring of the skin in which the scarring process interferes with normal renewal), psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination), and baldness (a condition in which terminally differentiated hair follicles (a tissue rich in Notch) fail to function properly). In another embodiment, a Therapeutic of the invention is used to treat degenerative or traumatic disorders of the sensory epithelium of the inner ear.

5.9. PROPHYLACTIC USES

5.9.1. MALIGNANCIES

The Therapeutics of the invention can be administered to prevent progression to a neoplastic or malignant state, including but not limited to those disorders listed in Table 1. Such administration is indicated where the Therapeutic is shown in assays, as described *supra*, to have utility for treatment or prevention of such disorder. Such prophylactic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has

occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, Basic Pathology, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79.) Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for 10 another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of 15 non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia 20 irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more 25 characteristics of a transformed phenotype, or of a malignant phenotype, displayed in vivo or displayed in vitro by a cell sample from a patient, can indicate the desirability of prophylactic/therapeutic administration of a Therapeutic of the invention. As mentioned supra, such characteristics of a 30 transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface 35 protein, etc. (see also id., at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of prophylactic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) is indicative of the desirability of prophylactic intervention.

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of a Therapeutic: a chromosomal translocation associated with a malignancy (e.g., the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (e.g., familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

In another specific embodiment, an Antagonist Therapeutic of the invention is administered to a human patient to prevent progression to breast, colon, or cervical cancer.

5.9.2. OTHER DISORDERS

In other embodiments, a Therapeutic of the invention can be administered to prevent a nervous system disorder described in Section 5.8.2, or other disorder (e.g., liver cirrhosis, psoriasis, keloids, baldness) described in Section 5.8.3.

5.10. DEMONSTRATION OF THERAPEUTIC OR PROPHYLACTIC UTILITY

The Therapeutics of the invention can be tested in vivo for the desired therapeutic or prophylactic activity. For example, such compounds can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For in vivo testing, prior to administration to humans, any animal model system known in the art may be used.

5.11. ANTISENSE REGULATION OF DELTA EXPRESSION

The present invention provides the therapeutic or prophylactic use of nucleic acids of at least six nucleotides that are antisense to a gene or cDNA encoding Delta or a portion thereof. "Antisense" as used herein refers to a nucleic acid capable of hybridizing to a portion of a Delta RNA (preferably mRNA) by virtue of some sequence complementarity. Such antisense nucleic acids have utility as Antagonist Therapeutics of the invention, and can be used in the treatment or prevention of disorders as described supra in Section 5.8 and its subsections.

The antisense nucleic acids of the invention can be oligonucleotides that are double-stranded or single-stranded, RNA or DNA or a modification or derivative thereof, which can be directly administered to a cell, or which can be produced intracellularly by transcription of exogenous, introduced sequences.

In a specific embodiment, the Delta antisense nucleic acids provided by the instant invention can be used for the treatment of tumors or other disorders, the cells of

which tumor type or disorder can be demonstrated (*in vitro* or *in vivo*) to express a *Delta* gene or a *Notch* gene. Such demonstration can be by detection of RNA or of protein.

The invention further provides pharmaceutical
5 compositions comprising an effective amount of the *Delta* antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described *infra* in Section 5.12. Methods for treatment and prevention of disorders (such as those described in Sections 5.8 and 5.9)
10 comprising administering the pharmaceutical compositions of the invention are also provided.

In another embodiment, the invention is directed to methods for inhibiting the expression of a *Delta* nucleic acid sequence in a prokaryotic or eukaryotic cell comprising
15 providing the cell with an effective amount of a composition comprising an antisense *Delta* nucleic acid of the invention.

Delta antisense nucleic acids and their uses are described in detail below.

20 5.11.1. DELTA ANTISENSE NUCLEIC ACIDS

The *Delta* antisense nucleic acids are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 50 oligonucleotides). In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 15
25 nucleotides, at least 100 nucleotides, or at least 200 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar
30 moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO 88/09810, published
35 December 15, 1988) or blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134, published April 25, 1988),

hybridization-triggered cleavage agents (see, e.g., Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents (see, e.g., Zon, 1988, Pharm. Res. 5:539-549).

In a preferred aspect of the invention, a Delta
5 antisense oligonucleotide is provided, preferably of single-stranded DNA. In a most preferred aspect, such an oligonucleotide comprises a sequence antisense to the sequence encoding an SH3 binding domain or a Notch-binding domain of Delta, most preferably, of human Delta. The
10 oligonucleotide may be modified at any position on its structure with substituents generally known in the art.

The Delta antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil,
15 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine,
20 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil,
25 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v),
30 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

In another embodiment, the oligonucleotide comprises at least one modified sugar moiety selected from the group including but not limited to arabinose,
35 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the oligonucleotide comprises at least one modified phosphate backbone selected

from the group consisting of a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

- 5 In yet another embodiment, the oligonucleotide is an α -anomeric oligonucleotide. An α -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641).

The oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

- 15 Oligonucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

- 25 In a specific embodiment, the *Delta* antisense oligonucleotide comprises catalytic RNA, or a ribozyme (see, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al., 1990, Science 247:1222-1225). In another embodiment, the oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

- In an alternative embodiment, the *Delta* antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector can be introduced *in vivo* such that it is taken up by a cell, within which cell the vector or a portion thereof is

transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the *Delta* antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it
5 can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence
10 encoding the *Delta* antisense RNA can be by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-
15 310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell* 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et
20 al., 1982, *Nature* 296:39-42), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a *Delta* gene, preferably a human *Delta* gene. However, absolute complementarity, although preferred,
25 is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded *Delta* antisense nucleic acids, a single strand of
30 the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a *Delta* RNA it may
35 contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a

tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

5.11.2. THERAPEUTIC UTILITY OF *DELTA*
ANTISENSE NUCLEIC ACIDS

5 The *Delta* antisense nucleic acids can be used to treat (or prevent) malignancies or other disorders, of a cell type which has been shown to express *Delta* or *Notch*. In specific embodiments, the malignancy is cervical, breast, or
10 colon cancer, or squamous adenocarcinoma. Malignant, neoplastic, and pre-neoplastic cells which can be tested for such expression include but are not limited to those described *supra* in Sections 5.8.1 and 5.9.1. In a preferred embodiment, a single-stranded DNA antisense *Delta*
15 oligonucleotide is used.

Malignant (particularly, tumor) cell types which express *Delta* or *Notch* RNA can be identified by various methods known in the art. Such methods include but are not limited to hybridization with a *Delta* or *Notch*-specific
20 nucleic acid (e.g. by Northern hybridization, dot blot hybridization, *in situ* hybridization), observing the ability of RNA from the cell type to be translated *in vitro* into *Notch* or *Delta*, immunoassay, etc. In a preferred aspect, primary tumor tissue from a patient can be assayed for *Notch*
25 or *Delta* expression prior to treatment, e.g., by immunocytochemistry or *in situ* hybridization.

Pharmaceutical compositions of the invention (see Section 5.12), comprising an effective amount of a *Delta* antisense nucleic acid in a pharmaceutically acceptable
30 carrier, can be administered to a patient having a malignancy which is of a type that expresses *Notch* or *Delta* RNA or protein.

The amount of *Delta* antisense nucleic acid which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or
35 condition, and can be determined by standard clinical techniques. Where possible, it is desirable to determine the

antisense cytotoxicity of the tumor type to be treated in vitro, and then in useful animal model systems prior to testing and use in humans.

In a specific embodiment, pharmaceutical compositions comprising Delta antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, it may be useful to use such compositions to achieve sustained release of the Delta antisense nucleic acids. In a specific embodiment, it may be desirable to utilize liposomes targeted via antibodies to specific identifiable tumor antigens (Leonetti et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2448-2451; Renneisen et al., 1990, J. Biol. Chem. 265:16337-16342).

15 5.12. THERAPEUTIC/PROPHYLACTIC
 ADMINISTRATION AND COMPOSITIONS

The invention provides methods of treatment (and prophylaxis) by administration to a subject of an effective amount of a Therapeutic of the invention. In a preferred aspect, the Therapeutic is substantially purified. The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.

Various delivery systems are known and can be used to administer a Therapeutic of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), construction of a Therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together

with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In another embodiment, the Therapeutic can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the Therapeutic can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise

(eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983);
5 see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of
10 the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

15 In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein Therapeutic, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that
20 it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in
25 linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., 1991, Proc. Natl. Acad. Sci. USA 88:1864-1868), etc. Alternatively, a nucleic acid Therapeutic can be introduced intracellularly and incorporated within host cell DNA for expression, by
30 homologous recombination.

In specific embodiments directed to treatment or prevention of particular disorders, preferably the following forms of administration are used:

<u>Disorder</u>	<u>Preferred Forms of Administration</u>
Cervical cancer	Topical
Gastrointestinal cancer	Oral; intravenous
5 Lung cancer	Inhaled; intravenous
Leukemia	Intravenous; extracorporeal
Metastatic carcinomas	Intravenous; oral
Brain cancer	Targeted; intravenous; intrathecal
Liver cirrhosis	Oral; intravenous
10 Psoriasis	Topical
Keloids	Topical
Baldness	Topical
Spinal cord injury	Targeted; intravenous; intrathecal
Parkinson's disease	Targeted; intravenous; intrathecal
15 Motor neuron disease	Targeted; intravenous; intrathecal
Alzheimer's disease	Targeted; intravenous; intrathecal

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel,

- sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides.
- Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the Therapeutic, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.
- In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for

injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental

agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

5

5.13. DIAGNOSTIC UTILITY

Delta proteins, analogues, derivatives, and subsequences thereof, Delta nucleic acids (and sequences complementary thereto), anti-Delta antibodies, have uses in
10 diagnostics. Such molecules can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting Delta expression, or monitor the treatment thereof. In particular, such an immunoassay is carried out by a method comprising
15 contacting a sample derived from a patient with an anti-Delta antibody under conditions such that immunospecific binding can occur, and detecting or measuring the amount of any immunospecific binding by the antibody. In a specific aspect, such binding of antibody, in tissue sections,
20 preferably in conjunction with binding of anti-Notch antibody can be used to detect aberrant Notch and/or Delta localization or aberrant levels of Notch-Delta colocalization in a disease state. In a specific embodiment, antibody to Delta can be used to assay in a patient tissue or serum
25 sample for the presence of Delta where an aberrant level of Delta is an indication of a diseased condition. Aberrant levels of Delta binding ability in an endogenous Notch protein, or aberrant levels of binding ability to Notch (or other Delta ligand) in an endogenous Delta protein may be
30 indicative of a disorder of cell fate (e.g., cancer, etc.) By "aberrant levels," is meant increased or decreased levels relative to that present, or a standard level representing that present, in an analogous sample from a portion of the body or from a subject not having the disorder.

35 The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays,

ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-
5 fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few.

Delta genes and related nucleic acid sequences and subsequences, including complementary sequences, and other toporythmic gene sequences, can also be used in hybridization
10 assays. Delta nucleic acid sequences, or subsequences thereof comprising about at least 8 nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or monitor conditions, disorders, or disease states associated with aberrant changes in Delta
15 expression and/or activity as described *supra*. In particular, such a hybridization assay is carried out by a method comprising contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to Delta DNA or RNA, under conditions such that hybridization can occur,
20 and detecting or measuring any resulting hybridization.

Additionally, since Delta binds to Notch, Delta or a binding portion thereof can be used to assay for the presence and/or amounts of Notch in a sample, e.g., in screening for malignancies which exhibit increased Notch
25 expression such as colon and cervical cancers.

6. A DELTA HOMOLOG IN THE CHICK IS EXPRESSED IN PROSPECTIVE NEURONS

As described herein, we have isolated and
30 characterized a chick Delta homologue, C-Delta-1. We show that C-Delta-1 is expressed in prospective neurons during neurogenesis, as new cells are being born and their fates decided. Our data in the chick, suggest that both the Delta/Notch signalling mechanism and its role in neurogenesis
35 have been conserved in vertebrates.

6.1. CLONING OF C-DELTA-1

We identified a chick Delta homologue, *C-Delta-1*, using the polymerase chain reaction (PCR) and degenerate oligonucleotide primers (Figures 1a, 1b and 2, SEQ ID NOS:1, 2, 3 and 4). *C-Delta-1* was cloned by PCR using the degenerate oligonucleotide primers TTCGGITT(C/T)ACITGGCCIGGIAC (SEQ ID NO:19) and TCIATGCAIGTICCCIC(A/G)TT (SEQ ID NO:20) which correspond to the fly Delta protein sequences FGFTWPQT (SEQ ID NO:21) and NGGTCID (SEQ ID NO:22), respectively (Vässin et al., 1987, EMBO J. 6:3431-3440; Kopczynski et al., 1988, Genes Dev. 2:1723-1735). The initial reaction used 50ng of first-strand oligo-d(T)-primed cDNA from stage 4-6 embryos, 1µg of each primer, 0.2mM dNTPs, 2U. of Taq polymerase, in 50µl of the supplied buffer (Perkin-Elmer). 40 cycles of amplification were performed at 94°C/30sec; 50°C/2min; 72°C/2min. Amplified DNA fragments were separated on an agarose gel, cloned in Bluescript pKS⁻ (Stratagene) and sequenced. Two Delta homologs were identified, one of which (*C-Delta-1*) is expressed in the nervous system. Of the homolog that is expressed in the nervous system, two variants were identified that differ at the carboxy-terminal end of the encoded protein due to an alternative splicing event at the 3' end of the *C-Delta-1* gene. One encoded protein has 12 extra amino acids at the carboxy-terminal end, relative to the other encoded protein. The sequence of the shorter encoded variant is set forth in SEQ ID NO:2. The longer variant encoded by SEQ ID NO:3 and identified by the amino acid sequence of SEQ ID NO:4, consists of the amino acid sequence of SEQ ID NO:2 plus twelve additional amino acids at the 3' end (SIPPGSRTSLGV). The longer variant was used in the experiments described below. When tested for biological activity by injection of RNA into *Xenopus* oocytes, each of the variants had the same biological activity.

DNA fragments corresponding to *C-Delta-1* were used to screen a stage 17 spinal cord cDNA library and several full-length clones were obtained and sequenced. We amplified

DNA fragments from chick *C-Notch-1* gene by similar methods (data not shown); partial sequence data and pattern of expression indicate close similarity to the rodent Notch-1 gene (Weinmaster et al., 1991, Development 113:199-205; 5 Weinmaster et al., 1992, Development 116:931-941; Lardelli & Lendahl, 1993, Exp. Cell Res. 204:364-372). Sequences were analyzed using the Wisconsin GCG set of programs. The GenBank Accession number for the Chick Delta-1 mRNA is U26590. The DNA sequence of *C-Delta-1* corresponds to a 10 protein of 722 amino acids, structurally homologous to *Drosophila* Delta (Figs. 3, 4) and clearly distinct from vertebrate homologs of the Delta-related Serrate protein, which we have also cloned (data not shown). *C-Delta-1* contains a putative transmembrane domain, a signal sequence 15 and 8 EGF-like repeats in its extracellular region (one repeat less than *Drosophila* Delta). The amino-terminal domain of *C-Delta-1* is closely related to a similar domain in the fly Delta protein, described as necessary and sufficient for *in vitro* binding to Notch (Muskavitch, 1994, Dev. Biol. 20 166:415-430). This conserved region includes the so-called DSL motif (Fig. 4) (Henderson et al., 1994, Development 120:2913-2924; Tax et al., 1994, Nature 368:150-154), shared by all known members of the family of presumed ligands of Notch-like proteins (Delta and Serrate in *Drosophila*; Lag-2 25 and Apx-1 in *Caenorhabditis elegans*) (Henderson et al., 1994, Development 120:2913-2924; Tax et al., 1994, Nature 368:150-154; Fleming et al., 1990, Genes Dev. 4:2188-2201; Thomas et al., 1991, Development 111:749-761; Mello et al., 1994, Cell 77:95-106). A second cysteine-rich N-terminal 30 region is conserved between the fly and chick proteins, but absent from the related *C. elegans* proteins (Fig. 4). The *Xenopus* Delta-1 homologue, *X-Delta-1* which encodes a protein that is 81% identical to *C-Delta-1* and shows all the above structural motifs (Fig. 3), has also been cloned. The 35 structural conservation between the chick and fly Delta proteins, including domains identified as critical for Notch binding (Muskavitch, 1994, Dev. Biol. 166:415-430), suggests

that C-Delta-1 functions as a ligand for a chick Notch protein, and that a Delta/Notch-mediated mechanism of lateral inhibition might operate in the chick.

5 6.2. C-DELTA-1 AND C-NOTCH-1 EXPRESSION
 CORRELATES WITH ONSET OF NEUROGENESIS

 During *Drosophila* neurogenesis, Delta is transiently expressed in neural precursors, inhibiting neighboring Notch-expressing cells from also becoming neural (Haenlin et al., 1990, Development 110:905-914; Kooh et al., 10 1993, Development 117:493-507). If C-Delta-1 acts similarly during chick neurogenesis, it should also be transiently expressed in neuronal precursor cells, while these are becoming determined. An analysis of C-Delta-1 expression in the developing CNS indicates that this is indeed the case. 15

 In summary, wholemount *in situ* hybridization was performed. Formaldehyde fixed embryos were treated with protease and refixed with 4% formaldehyde/0.1% glutaraldehyde. Hybridization with DIG-labelled RNA probes was performed under stringent conditions (1.3xSSC, 50% 20 formamide, 65°C, pH5) in a buffer containing 0.2% Tween-20 and 0.5% CHAPS. Washed embryos were treated with Boehringer Blocking Reagent and incubated overnight in alkaline phosphatase-coupled anti-DIG antibody. After extensive washes, embryos were stained from 30min to overnight. The 25 embryo in Figure 5e was wax-sectioned after hybridization.

 C-Delta-1 expression in the neural plate is first detected at stage 6-7 (31h, 0/1 somite), in scattered cells just anterior to the presomitic mesoderm (Fig. 5b, 5c). This 30 region gives rise to the mid/posterior hindbrain, where the earliest differentiated CNS neurons are first detected by a neurofilament antibody at stage 9 (31h, 7-9 somites) (Sechrist & Bronner-Fraser, 1991, Neuron 7:947-963), 6h after the initial C-Delta-1 expression (Table 2).

35

		TABLE 2		
		Hamburger-Hamilton Stage (nominal age in h; somite nos.)		
5	Neural tube domains	End final S-phase	Initial <i>C-Delta-1</i> expression	Initial NF expression
	Mid/posterior Hindbrain	4 (19h; 0)	6 (24h; 0)	9 (31h; 7-9)
10	Spinal cord, somites 5-8	6 (24h; 0)	8 (28h; 4-6)	10 (36h; 10-12)
	Forebrain/ Midbrain	7 (25h; 1-3)	8 (28h; 4-6)	10 (36h; 10-12)
	Spinal cord, somites 9-12	8 (28h; 4-6)	9 (31h; 7-9)	11 (43h; 13-15)
15				

As neurogenesis proceeds, expression of *C-Delta-1* continues to foreshadow the spatio-temporal pattern of neuronal differentiation (Table 2), spreading posteriorly along the spinal cord and anteriorly into the midbrain and forebrain (Fig 5d, 5e). For example, the most posterior expressing cells in the stage 8 spinal cord are at the level of the prospective 6th somite, 6-8h before the first neurons at that level express neurofilament antigen (Sechrist & Bronner-Fraser, 1991, Neuron 7:947-963) (Table 2). Table 2 shows that the appearance of *C-Delta-1* expression closely follows the withdrawal of the first neuronal precursors from the division cycle and precedes the appearance of neurofilament (NF) antigen in the resultant differentiating neurons. Mid-hindbrain comprises rhombomeres 4-6, the level of the otic primordium; posterior hindbrain includes rhombomeres 7 and 8, and somites 1-4. Data for the timing of withdrawal from cell-division and for neurofilament expression are taken from Sechrist et al., 1991, Neuron 7:947-963. In all cases, *C-Delta-1* is expressed in scattered cells within domains of uniform *C-Notch-1* expression (Fig. 5a).

6.3. LOCALIZATION AND TIME-COURSE EXPRESSION OF C-DELTA-1

The localization and time-course of *C-Delta-1* expression indicate that the gene is switched on at an early step in neurogenesis, and that the cells expressing *C-Delta-1* are prospective neurons that have not yet begun to display differentiation markers. To test this hypothesis, we made use of the observations of Sechrist and Bronner-Fraser (Sechrist & Bronner-Fraser, 1991, Neuron 7:947-963) that prospective neurons are the only non-cycling cells in the early neural tube. They finish their final S phase 11-15h before expressing neurofilament antigen (Table 2) and their nuclei, after completing a last mitosis, adopt a characteristic location near the basal surface of the neuroepithelium, where all the other cell nuclei are in S-phase (Sechrist & Bronner-Fraser, 1991, Neuron 7:947-963; Martin & Langman, 1965, J. Embryol. Exp. Morphol. 14:23-35) (Fig. 6a). We labelled stage 7-9 embryos with bromodeoxyuridine (BrdU), and double-stained for BrdU incorporation and *C-Delta-1* expression. 95% of the *C-Delta-1*-expressing cells were unlabelled, with their nuclei predominantly located near the basal surface, where most other nuclei were BrdU-labelled (Fig. 6b, 6c). 75µl 0.1mM BrdU in PBS was dropped onto stage 7-9 embryos which were incubated at 38°C for 2-4h before fixation for *in situ* hybridization. 15µm cryostat sections were hybridized with DIG-labelled RNA probes, essentially according to the method of Strähle et al. (Strähle et al., 1994, Trends In Genet. Sci. 10:75-76). After staining, slides were washed in PBS, and processed for BrdU immunodetection (Biffo et al., 1992, Histochem. Cytochem. 40:535-540). Anti-BrdU (1:1000; Sigma) was detected using FITC-coupled goat anti-mouse secondary antibody (Cappel). *C-Delta-1* expression was examined by DIC microscopy, and BrdU-labelling by conventional and confocal fluorescence microscopy. These results imply that *C-Delta-1* is expressed in cells that have withdrawn from the cell cycle and must indeed be prospective neurons. The few BrdU⁺/*C-*

Delta-1 cells have their nuclei outside the basal zone; these may be cells that finished their final S-phase soon after exposure to BrdU, moved apically to complete their final mitosis, and switched on *C-Delta-1* expression. *C-Delta-1* is
5 also expressed in the later neural tube and peripheral nervous system. Again, the timing of expression and the location of the expressing cells imply that they are neuronal precursors that have not yet begun to differentiate (data not shown). Thus, *C-Delta-1* expression appears to be the
10 earliest known marker for prospective neurons.

In addition, the transcription pattern of both *C-Delta-1* and *C-Serrate-1* overlap that of *C-Notch-1* in many regions of the embryo (data not shown) which suggest that *C-Notch-1*, like Notch in *Drosophila*, is a receptor for both
15 proteins. In particular, all three genes are expressed in the neurogenic region of the developing central nervous system, and here a striking relationship is seen: the expression of both *C-Serrate-1* and *C-Delta-1* is confined to the domain of *C-Notch-1* expression; but within this domain,
20 the regions of *C-Serrate-1* and *C-Delta-1* are precisely complementary. The overlapping expression patterns suggest conservation of their functional relationship with Notch and imply that development of the chick and in particular the central nervous system involves the concerted interaction of
25 *C-Notch-1* with different ligands at different locations.

6.4. DISCUSSION

The *Xenopus* homolog of *C-Delta-1* has been cloned in a similar manner. In brief, a PCR fragment of *X-Delta-1* was
30 isolated and sequenced. This fragment was then used to identify the full length clone of *X-Delta-1*. The *X-Delta-1* expression pattern was studied. It was shown that *X-Delta-1* is expressed in scattered cells in the domain of the neural plate where primary neuronal precursors are being generated,
35 suggesting that the cells expressing *X-Delta-1* are the prospective primary neurons. In addition, *X-Delta-1* is also expressed at other sites and times of neurogenesis, including

the anterior neural plate and neurogenic placodes and later stages of neural tube development when secondary neurons are generated. Ectopic X-Delta-1 activity inhibited production of primary neurons; interference with endogenous X-Delta-1 activity resulted in overproduction of primary neurons.

These results show that X-Delta-1 mediates lateral inhibition delivered by prospective neurons to adjacent cells. It was shown that ectopic expression of X-Delta-1 in *Xenopus* eggs suppresses primary neurogenesis, and that ectopic expression of a truncated X-Delta-1 protein which retains only two amino acids of the cytoplasmic domain interferes with endogenous signalling and leads to extra cells developing as neuronal precursors. (Chitnis et al., *Nature* (in press). Preliminary evidence indicates that C-Delta-1 has a similar inhibitory action when expressed in *Xenopus* embryos (data not shown).

We propose that C-Delta-1, like its *Drosophila* and *Xenopus* counterparts, mediates lateral inhibition throughout neurogenesis to restrict the proportion of cells that, at any time, become committed to a neural fate. C-Delta-1 is generally expressed during neurogenesis in many other sites, in both the CNS and PNS, and, for example, the developing ear. It has been shown in the CNS that C-Notch is expressed in the ventricular zone of the E5 chick hindbrain, in dividing cells adjacent to the lumen of the neural tube. C-Delta-1 is expressed in the adjacent layer of cells, which have stopped dividing and are becoming committed as neuronal precursor cells. Thus, Delta/Notch signalling could act here, as in other neural tissues, to maintain a population of uncommitted cycling neuronal stem cells.

7. ISOLATION AND CHARACTERIZATION OF A MOUSE *DELTA* HOMOLOG

A mouse *Delta* homolog, termed M-Delta-1, was isolated as follows:

Mouse *Delta*-1 gene

Tissue Origin: 8.5 and 9.5-day mouse embryonic RNA

Isolation Method:

a) random primed cDNA against above RNA

b) PCR of above cDNA using

PCR primer 1: GGITTCACITGGCCIGGIACNTT

(SEQ ID NO:23) [encoding GFTWPGTF (SEQ ID NO:24), a region which is specific for Delta-, not Serrate-like proteins]

PCR primer 2:

GTICCCICG(G/A)TT(C/T)TT(G/A)CAIGG(G/A)TT

(SEQ ID NO:25) [encoding NPCKNGGT (SEQ ID NO:26), a sequence present in many of the EGF-like repeats]

Amplification conditions: 50 ng cDNA, 1 µg each primer, 0.2 mM dNTP's, 1.8 U Taq (Perkin-Elmer) in 50 µl of supplied buffer. 40 cycles of: 94°C/30 sec, 45°C/2 min, 72°C/1 min extended by 2 sec each cycle.

The amplified fragment was an approximately 650 base pair fragment which was partially sequenced to determine its relationship to C-Delta-1.

c) a mouse 11.5 day cDNA library (Clontech) was screened. Of several positive clones, one (pMDL2; insert size approximately 4 kb) included the complete protein-coding region whose DNA sequence was completely determined.

Figure 7 (SEQ ID NO:11) shows the nucleotide sequence of the isolated clone containing M-Delta-1 DNA.

Figure 8 (SEQ ID NO:12) shows the predicted amino acid sequence of M-Delta-1.

Figure 9 shows and amino acid alignment of the predicted amino acid sequences for M-Delta-1 and C-Delta-1.

Identical amino acids are boxed showing the extensive sequence homology. The consensus sequence is shown below (SEQ ID NO:13).

Expression pattern: The expression pattern was determined to be essentially the same as that observed for C-Delta-1, in particular, in the presomitic mesoderm, central nervous system, peripheral nervous system, and kidney.

8. ISOLATION AND CHARACTERIZATION OF A HUMAN DELTA HOMOLOG

A human Delta-1 homolog, termed H-Delta-1 (HD1), was isolated as follows:

5 A human genomic library with inserts ranging in size from 100-150 kb was probed with an EcoRI fragment of the mouse Delta-1 (M-Delta-1) gene. From the library a genomic human PAC clone was isolated which hybridized to the EcoRI fragment. Next, two degenerate oligonucleotides were used to amplify by PCR a fragment of the genomic human PAC clone.
10 The degenerate oligos were:

5' ACIATGAA(C/T)AA(C/T)CTIGCIAA(C/T)TG (SEQ ID NO:27)

[encoding TMNNLANC (SEQ ID NO:28)] and

3' AC(A/G)TAIACIGA(C/T)TG(A/G)TA(C/T)TTIGT (SEQ ID NO:29)

15 [encoding TKYQSVYV (SEQ ID NO:30) or

3' GC(A/G/T)ATIAC(A/G)CA(C/T)TC(A/G)TC(C/T)TT(C/T)TC

(SEQ ID NO:31) [encoding EKDECVIA (SEQ ID NO:32)].

On the basis of the cDNA sequences for chicken and mouse Delta-1, it was expected that fragments of approximately 354 and 387 base pairs would be isolated, using the 5' and the
20 two different 3' oligos, respectively. In fact, however, two single isolates of 525 base pairs and another that was 30 base pairs smaller, as expected, were obtained. The larger isolate was sequenced by dideoxy sequencing. The nucleotide sequence is shown in Figure 10 (SEQ ID NO:14). Also shown in
25 Figure 10 are the predicted amino acid sequences of the amplified DNA fragment (SEQ ID NOS:15, 16, 17) for the three different readings frames. Due to sequencing errors, the full uninterrupted sequence between both primers was not identified. As a consequence, one cannot predict the amino
30 acid sequence directly from the DNA sequence obtained.

However, Figure 11 shows the amino acid sequence homology between human Delta-1 (top line) (SEQ ID NO:18) and chick Delta-1 (bottom line) as determined by eye. Because of the
35 sequencing errors, the homology was obtained by switching amongst the three different reading frames to identify the homologous regions.

Using the larger isolate (SEQ ID NO:14) as probe, a human fetal brain plasmid library (Clontech) was screened in an attempt to isolate full-length H-Delta-1 (HD1) genes. This yielded four positive plaques. Two of these positives (HD13 and HD124) survived rescreening and reacted positively with a large human genomic fragment on a Southern Blot. These positive clones were subcloned by digesting with *EcoRI* and ligating the fragments into a Bluescript KS⁻ vector. The nucleotide sequences of the inserts were obtained by dideoxy sequencing using T3 and T7 primers. The results showed that HD124 was homologous to chicken Delta-1 at both ends; however, one end of HD13 showed no homology. Restriction digestions with a panel of enzymes showed very similar patterns between the two clones, each of which had an insert of about 2 kb, but with differences at the 3' end of HD13.

HD13 and HD124 were cut with *BstXI*, *XbaI*, *HindIII* and *XhoI* and the restriction fragments were inserted into Bluescript KS⁻, and then sequenced as described above to obtain internal sequence. The sequence that was obtained represents the 3' about 2000 bases of HD1, extending into the 3' non-coding region. HD13 is contained within HD124; however, the added sequence at the 5' end of HD13 is likely due to a cloning artifact.

Since the sequence thus obtained did not contain the 5' end of HD1, HD124 was used as a probe for subsequent hybridizations in a T cell library and in another fetal brain library (Lambda-Zap, Stratagene). A screen of the T cell library resulted in no positives. However, screening the Lambda-Zap library resulted in two positive clones, HD113 and HD118. These clones were inserted into a Bluescript KS⁻ vector using *EcoRI* as described above. The inserts were digested with a panel of restriction enzymes for comparison with HD13 and HD124, and the 5' and 3' ends were sequenced using T3 and T7 primers. HD113 was determined to be only a small piece of cDNA that when sequenced showed no homology to any known Delta. However, HD118 was 3 kb in length, and included the entire sequence of HD124 with additional 5'

sequences. A set of clones were isolated using nested deletions from HD118; these clones were then subjected to dideoxy sequencing using an automated sequencer. Figure 12A presents the partial nucleotide contig sequence (SEQ ID NO:33) of human *Delta* obtained from clone HD118. Due to sequencing errors, the full uninterrupted nucleotide sequence of human *Delta* was not determined. Figure 12B shows the partial nucleotide contig sequence (SEQ ID NO:33) of human *Delta* (top line), with the predicted amino acid sequence in three different reading frames presented below, the second line being reading frame 1 (SEQ ID NO:34), the third line being reading frame 2 (SEQ ID NO:35), and the fourth line being reading frame 3 (SEQ ID NO:36).

Sequence homology was determined by eye using the mouse *Delta*-1 amino acid sequence. The sequences with the greatest degree of homology to the mouse amino acid sequence are boxed in Figure 12B, and represent the predicted amino acid sequence of human *Delta*-1. The composite resulting amino acid sequence is shown in Figure 14. (In Figure 14, the various uninterrupted portions of the human *Delta* sequence are assigned respectively, SEQ ID NOS:39 through 65.) Note that due to sequencing errors, the reading frame with the greatest homology is not the same throughout the sequence and shifts at positions where there are errors in the sequence.

Further, the homology determined by eye to chicken and mouse *Delta* indicates that the amino acid sequence deduced from the determined human *Delta* nucleotide sequence contains all but about the N-terminal 100-150 amino acids of human *Delta*-1.

Figure 13 presents the nucleotide sequence of mouse *Delta*-1 (top line, SEQ ID NO:37) and the contig nucleotide sequence of human *Delta*-1 as depicted in Figures 12A and 12B (second line, SEQ ID NO:33) and the nucleotide consensus sequence between mouse and human *Delta* (third line, SEQ ID NO:38).

Using probes containing the human Delta 5' nucleotide sequences presented in Figure 12A, cDNA libraries are probed to isolate the 5' end of the human Delta gene. Primary positive clones are obtained and then confirmed as 5 secondary positives. The secondary positives are purified and grown further. The DNA is then isolated and subcloned for sequencing.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, 10 various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

15 Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

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WHAT IS CLAIMED IS:

1. A purified vertebrate Delta protein.
- 5 2. The protein of claim 1 which is a human protein.
3. The protein of claim 1 which is a mammalian protein.
- 10 4. The protein of claim 1 which comprises the amino acid sequence substantially as set forth in amino acid numbers 1-722 of SEQ ID NO:12.
- 15 5. A purified derivative or analog of the protein of claim 1, which is able to display one or more functional activities of a Delta protein.
6. A purified derivative or analog of the protein
20 of claim 2, which is able to display one or more functional activities of a human or *D. melanogaster* Delta protein.
7. The derivative or analog of claim 5 which is able to be bound by an antibody directed against a human or
25 *D. melanogaster* Delta protein.
8. A purified fragment of the protein of claim 2, which is able to be bound by an antibody directed against a human Delta protein.
- 30 9. A molecule comprising the fragment of claim 8.
10. A purified fragment of the protein of claim 2 which is able to display one or more functional activities of
35 a human Delta protein.

11. A purified fragment of a vertebrate Delta protein comprising a domain of the protein selected from the group consisting of the extracellular domain, DSL domain, domain amino-terminal to the DSL domain, epidermal growth factor-like repeat domain, transmembrane domain, and intracellular domain.

12. A purified fragment of a Delta protein comprising the membrane-associated region of the protein.

13. A purified fragment of a Delta protein comprising an epidermal growth factor-homologous repeat of the protein.

14. The fragment of claim 11 in which the Delta protein is a human Delta protein.

15. A purified fragment of a vertebrate Delta protein comprising a region homologous to a Notch protein or a Delta protein, and consisting of at least six amino acids.

16. A purified fragment of a vertebrate Delta protein comprising the region of the protein with the greatest homology over an identical number of amino acids to amino acid numbers 1-722 as shown in Figure 8 (SEQ ID NO:12).

17. A chimeric protein comprising a fragment of a vertebrate Delta protein consisting of at least 20 amino acids fused via a covalent bond to an amino acid sequence of a second protein, in which the second protein is not the Delta protein.

18. The chimeric protein of claim 17 in which the fragment of a vertebrate Delta protein is a fragment capable of being bound by an anti-Delta antibody.

19. The chimeric protein of claim 18 in which the Delta protein is a human protein.

20. The chimeric protein of claim 19 which is able to display one or more functional activities of a Delta protein.

21. A purified fragment of a vertebrate Delta protein which (a) is capable of being bound by an anti-Delta antibody; and (b) lacks the transmembrane and intracellular domains of the protein.

22. A purified fragment of a vertebrate Delta protein which (a) is capable of being bound by an anti-Delta antibody; and (b) lacks the extracellular domain of the protein.

23. A purified fragment of a vertebrate Delta protein which is able to bind to a Notch protein.

24. The fragment of claim 23, which lacks the epidermal growth factor-like repeats of the Delta protein.

25. The fragment of claim 23 in which the Delta protein is a human Delta protein.

26. The fragment of claim 23, which is a fragment of SEQ ID NO:18.

27. A molecule comprising the fragment of claim 23.

28. The fragment of claim 11 or 21 in which the Delta protein is a human Delta protein.

29. An antibody which is capable of binding the Delta protein of claim 1, and which does not bind to a *Drosophila* Delta protein.

5 30. An antibody which is capable of binding the Delta protein of claim 2, and which does not bind to a *Drosophila* Delta protein.

 31. The antibody of claim 1 which is monoclonal.

10

 32. A molecule comprising a fragment of the antibody of claim 31, which fragment is capable of binding a Delta protein.

15 33. An isolated nucleic acid comprising a nucleotide sequence encoding a vertebrate Delta protein.

 34. The nucleic acid of claim 33 which is DNA.

20 35. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence of claim 33.

 36. An isolated nucleic acid comprising a
25 nucleotide sequence encoding the Delta protein of claim 2.

 37. An isolated nucleic acid comprising a fragment of a vertebrate Delta gene consisting of at least 50 nucleotides.

30

 38. An isolated nucleic acid comprising a nucleotide sequence encoding the fragment of claim 10.

 39. An isolated nucleic acid comprising a
35 nucleotide sequence encoding the fragment of claim 11.

40. An isolated nucleic acid comprising a nucleotide sequence encoding the fragment of claim 23.

41. An isolated nucleic acid comprising a
5 nucleotide sequence encoding a protein, said protein comprising amino acid numbers 1-175 of the human Delta sequence depicted in Figure 11 (SEQ ID NO:18).

42. An isolated nucleic acid comprising a
10 nucleotide sequence encoding the protein of claim 17.

43. A recombinant cell containing the nucleic acid of claim 33.

15 44. A recombinant cell containing the nucleic acid of claim 39.

45. A recombinant cell containing the nucleic acid of claim 41.

20

46. A method of producing a vertebrate Delta protein comprising growing a recombinant cell containing the nucleic acid of claim 33 such that the encoded vertebrate Delta protein is expressed by the cell, and recovering the
25 expressed Delta protein.

47. A method of producing a vertebrate Delta protein comprising growing a recombinant cell containing the nucleic acid of claim 41 such that the encoded Delta protein
30 is expressed by the cell, and recovering the expressed Delta protein.

48. A method of producing a protein comprising a fragment of a vertebrate Delta protein, which method
35 comprises growing a recombinant cell containing the nucleic acid of claim 39 such that the encoded protein is expressed by the cell, and recovering the expressed protein.

49. The product of the process of claim 46.

50. The product of the process of claim 47.

5 51. The product of the process of claim 48.

52. A pharmaceutical composition comprising a therapeutically effective amount of a Delta protein; and a pharmaceutically acceptable carrier.

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53. The composition of claim 52 in which the Delta protein is a human Delta protein.

54. A pharmaceutical composition comprising a
15 therapeutically effective amount of the fragment of claim 11; and a pharmaceutically acceptable carrier.

55. A pharmaceutical composition comprising a therapeutically effective amount of the fragment of claim 23;
20 and a pharmaceutically acceptable carrier.

56. A pharmaceutical composition comprising a therapeutically effective amount of a derivative or analog of a Delta protein, which derivative or analog is characterized
25 by the ability to bind to a Notch protein or to a molecule comprising the epidermal growth factor-like repeats 11 and 12 of a Notch protein; and a pharmaceutically acceptable carrier.

30 57. A pharmaceutical composition comprising a therapeutically effective amount of the nucleic acid of claim 33; and a pharmaceutically acceptable carrier.

58. A pharmaceutical composition comprising a
35 therapeutically effective amount of the nucleic acid of claim 35; and a pharmaceutically acceptable carrier.

59. A pharmaceutical composition comprising a therapeutically effective amount of the nucleic acid of claim 39; and a pharmaceutically acceptable carrier.

5 60. A pharmaceutical composition comprising a therapeutically effective amount of an antibody which binds to a Delta protein; and a pharmaceutically acceptable carrier.

10 61. A pharmaceutical composition comprising a therapeutically effective amount of a fragment or derivative of an antibody to a Delta protein containing the binding domain of the antibody; and a pharmaceutically acceptable carrier.

15 62. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a Delta protein or
20 derivative thereof which is able to bind to a Notch protein.

63. The method according to claim 62 in which the disease or disorder is a malignancy characterized by increased Notch activity or increased expression of a Notch
25 protein or of a Notch derivative capable of being bound by an anti-Notch antibody, relative to said Notch activity or expression in an analogous non-malignant sample.

64. The method according to claim 62 in which the
30 disease or disorder is selected from the group consisting of cervical cancer, breast cancer, colon cancer, melanoma, seminoma, and lung cancer.

65. The method according to claim 62 in which the
35 subject is a human.

66. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired a therapeutically effective amount of a molecule, in which the
5 molecule is an oligonucleotide which (a) consists of at least six nucleotides; (b) comprises a sequence complementary to at least a portion of an RNA transcript of a *Delta* gene; and (c) is hybridizable to the RNA transcript.

10 67. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired an effective amount of the nucleic acid of claim 33 or 39.

15 68. A method of treating or preventing a disease or disorder in a subject comprising administering to a subject in which such treatment or prevention is desired an effective amount of the antibody of claim 30.

20 69. The method according to claim 62 in which the disease or disorder is a disease or disorder of the central nervous system.

70. An isolated oligonucleotide consisting of at
25 least six nucleotides, and comprising a sequence complementary to at least a portion of an RNA transcript of a *Delta* gene, which oligonucleotide is hybridizable to the RNA transcript.

30 71. A pharmaceutical composition comprising the oligonucleotide of claim 70; and a pharmaceutically acceptable carrier.

72. A method of inhibiting the expression of a
35 nucleic acid sequence encoding a *Delta* protein in a cell comprising providing the cell with an effective amount of the oligonucleotide of claim 70.

73. A method of diagnosing a disease or disorder characterized by an aberrant level of Notch-Delta protein binding activity in a patient, comprising measuring the ability of a Notch protein in a sample derived from the
5 patient to bind to a Delta protein, in which an increase or decrease in the ability of the Notch protein to bind to the Delta protein, relative to the ability found in an analogous sample from a normal individual, indicates the presence of the disease or disorder in the patient.

10

74. A method of diagnosing a disease or disorder characterized by an aberrant level of Delta protein in a patient, comprising measuring the level of Delta protein in a sample derived from the patient, in which an increase or
15 decrease in the level of Delta protein, relative to the level of Delta protein found in an analogous sample from a normal individual, indicates the presence of the disease or disorder in the patient.

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75. A purified human protein which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

25

76. The fragment of claim 8 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of
30 said sequence.

77. The fragment of claim 10 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A
35 (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

78. The fragment of claim 14 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of
5 said sequence.

79. The fragment of claim 25 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A
10 (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

80. The fragment of claim 10 or 25, which is a fragment of SEQ ID NO:39.
15

81. The fragment of claim 28 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of
20 said sequence.

82. An isolated nucleic acid comprising the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33).

83. An isolated nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33).
25

84. A purified protein comprising at least a
30 portion of a human Delta amino acid sequence, said portion selected from the group consisting of amino acid numbers 1-192 depicted in Figure 14 (SEQ ID NO:39), amino acid numbers 205-213 depicted in Figure 14 (SEQ ID NO:43), amino acid numbers 214-370 depicted in Figure 14 (SEQ ID NO:44), amino
35 acid numbers 371-382 depicted in Figure 14 (SEQ ID NO:45), amino acid numbers 394-418 depicted in Figure 14 (SEQ ID NO:49), amino acid numbers 419-428 depicted in Figure 14 (SEQ

ID NO:50), amino acid numbers 443-458 depicted in Figure 14 (SEQ ID NO:52), amino acid numbers 459-469 depicted in Figure 14 (SEQ ID NO:53), amino acid numbers 470-495 depicted in Figure 14 (SEQ ID NO:54), amino acid numbers 496-508 depicted in Figure 14 (SEQ ID NO:55), and amino acid numbers 516-519 depicted in Figure 14 (SEQ ID NO:59).

85. The protein of claim 84 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

86. A purified protein which is encoded by a first nucleic acid hybridizable under stringent conditions to a second nucleic acid having a nucleotide sequence comprising a sequence selected from the group consisting of nucleotide numbers 60-634 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 746-772 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 775-1245 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1249-1284 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1415-1489 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1493-1522 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1526-1567 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1570-1618 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1622-1653 depicted in Figure 12B (SEQ ID NO:33), nucleotide numbers 1658-1735 depicted in Figure 12B (SEQ ID NO:33), and nucleotide numbers 1739-1777 depicted in Figure 12B (SEQ ID NO:33).

87. The protein of claim 2 which comprises a portion of the human Delta amino acid sequence set forth in Figure 14, said portion selected from the group consisting of amino acid numbers 1-192 (SEQ ID NO:39), amino acid numbers 205-213 (SEQ ID NO:43), amino acid numbers 214-370 (SEQ ID NO:44), amino acid numbers 371-382 (SEQ ID NO:45), amino acid

numbers 394-418 (SEQ ID NO:49), amino acid numbers 419-428 (SEQ ID NO:50), amino acid numbers 443-458 (SEQ ID NO:52), amino acid numbers 459-469 (SEQ ID NO:53), amino acid numbers 470-495 (SEQ ID NO:54), amino acid numbers 496-508 (SEQ ID NO:55), and amino acid numbers 516-519 (SEQ ID NO:59).

88. The protein of claim 75 in which the first nucleic acid is hybridizable to the second nucleic acid under conditions of high stringency.

10

89. The fragment of claim 76, 77 or 78 in which the first nucleic acid is hybridizable to the second nucleic acid under conditions of high stringency.

15

90. An isolated nucleic acid hybridizable under conditions of high stringency to a nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

20

91. The nucleic acid of claim 90 which comprises a cDNA sequence hybridizable under conditions of high stringency to a nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

25

92. An isolated nucleic acid comprising a nucleotide sequence complementary to a cDNA sequence hybridizable under conditions of high stringency to a nucleic acid having the nucleotide sequence depicted in Figure 12A (SEQ ID NO:33) or having an at least 50 nucleotide portion of said sequence.

93. A purified human protein which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 10 (SEQ ID NO:14) or having an at least 50 nucleotide portion of said sequence.

94. The fragment of claim 8 which is encoded by a first nucleic acid that is hybridizable to a second nucleic acid having the nucleotide sequence depicted in Figure 10 (SEQ ID NO:14) or having an at least 50 nucleotide portion of 5 said sequence.

95. An isolated nucleic acid hybridizable under conditions of high stringency to a nucleic acid having the nucleotide sequence depicted in Figure 10 (SEQ ID NO:14) or 10 having an at least 50 nucleotide portion of said sequence.

96. An isolated nucleic acid hybridizable under conditions of high stringency to a nucleic acid having the consensus nucleotide sequence depicted in Figure 13 (SEQ ID 15 NO:38) or having an at least 50 nucleotide portion of said sequence.

97. A purified protein encoded by a first nucleic acid hybridizable to a second nucleic acid having the 20 consensus nucleotide sequence depicted in Figure 13 (SEQ ID NO:38) or having an at least 50 nucleotide portion of said sequence.

98. An isolated nucleic acid comprising a 25 nucleotide sequence that is complementary to the nucleotide sequence of the nucleic acid of claim 92 or 96.

30

35

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GAATTCGGCAGAGGTTTTTTTTTTTTTCCCTCTTTCTTTCTTTCTTTGCG
1 -----+-----+-----+-----+-----+-----+ 60

ATCCGAAAGAGCTGTCAGCCGCCGCCGGGCTGCACCTAAAGGCGTCGGTAGGGGATAAC
61 -----+-----+-----+-----+-----+-----+ 120

AGTCAGAGACCCTCCTGAAAGCAGGAGACGGGACGGTACCCCTCCGGCTCTGCGGGGCGG
121 -----+-----+-----+-----+-----+-----+ 180

CTGCGGCCCTCCGTTCTTTCCCTCCCGAGAGACACTCTTCCTTTCCCCCACGAAG
181 -----+-----+-----+-----+-----+-----+ 240

ACACAGGGGCAGGAACGCGAGCGCTGCCCCCTCCGCATGGGAGGCCGCTTCTGCTGACG
241 -----+-----+-----+-----+-----+-----+ 300

CTCGCCCTCCTCTCGGCGCTGCTGTGCCGCTGCCAGGTTGACGGCTCCGGGGTGTTGAG
301 -----+-----+-----+-----+-----+-----+ 360

CTGAAGCTGCAGGAGTTTGTCAACAAGAAGGGCTGCTCAGCAACCGCAACTGCTGCCGG
361 -----+-----+-----+-----+-----+-----+ 420

GGGGGCGGCCCCGAGGCGCCGGGCAGCAGCAGTGGCACTGCAAGACCTTCTTCGCGTC
421 -----+-----+-----+-----+-----+-----+ 480

TGCCTGAAGCACTACCAGGCCAGCGTCTCCCCGAGCCGCCCTGCACCTACGGCAGCGCC
481 -----+-----+-----+-----+-----+-----+ 540

ATCACCCCGTCTCGGCGCCAACCTCCTTCAGCGTCCCCGACGGCGGGCGGCGCCGAC
541 -----+-----+-----+-----+-----+-----+ 600

CCCGCCTTCAGCAACCCCATCCGCTTCCCCTCGGCTTACCTGGCCCGGCACCTTCTCG
601 -----+-----+-----+-----+-----+-----+ 660

CTCATCATCGAGGCTCTGCACACCGACTCCCCGACGACCTCACCACAGAAAACCCGAG
661 -----+-----+-----+-----+-----+-----+ 720

CGCCTCATCAGCCGCTGGCCACCCAGAGGCACCTGGCGGTGGGCGAGGAGTGGTCCAG
721 -----+-----+-----+-----+-----+-----+ 780

GACCTGCACAGCAGCGGCCGACCGACCTCAAGTACTCTATCGCTTTGTGTGTATGAG
781 -----+-----+-----+-----+-----+-----+ 840

FIG. 1A1
SUBSTITUTE SHEET (RULE 26)

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CACTACTACGGGGAAGGCTGCTCTGTCTTCTGCCGGCCCCGTGACGACCGCTTCGGTCAC
841 -----+-----+-----+-----+-----+-----+ 900

TTCACCTGTGGAGAGCGTGGCGAGAAGGTCTGCAACCCAGGCTGGAAGGGCCAGTACTGC
901 -----+-----+-----+-----+-----+-----+ 960

ACTGAGCCGATTTGCTTGCCTGGGTGTGACGAGCAGCACGGCTTCTGCGACAAACCTGGG
961 -----+-----+-----+-----+-----+-----+ 1020

GAATGCAAGTGCAGAGTGGGTTGGCAGGGGCGGTACTGTGACGAGTGCATCCGATACCCA
1021 -----+-----+-----+-----+-----+-----+ 1080

GGCTGCCTGCACGGTACCTGTGACGAGCCATGGCAGTGCAACTGCCAGGAAGGCTGGGGC
1081 -----+-----+-----+-----+-----+-----+ 1140

GGCCTTTTCTGCAACCAGGACCTGAACTACTGCACTCACCACAAGCCATGCAAGAATGGT
1141 -----+-----+-----+-----+-----+-----+ 1200

CGGTGTACGTGGTTGTGGCCAGTCCCCTCGATGTGAACAAGAACGGCTGGACCCATGTGT
1201 -----+-----+-----+-----+-----+-----+ 1260

GGCTCCAGCTGCGAGATTGAAATCAACGAATGTGATGCCAACCCTTGCAAGAATGGTGGA
1261 -----+-----+-----+-----+-----+-----+ 1320

AGCTGCACGGATCTCGAGAACAGCTATTCTGTACCTGCCCCCAGGCTTCTATGGTAAA
1321 -----+-----+-----+-----+-----+-----+ 1380

AACTGTGAGCTGAGTGCAATGACTTGTGCTGATGGACCGTGCTTCAATGGAGGGCGATGC
1381 -----+-----+-----+-----+-----+-----+ 1440

ACTGACAACCCTGATGGTGGATACAGCTGCCGCTGCCCACTGGGTTATTCTGGGTTCAAC
1441 -----+-----+-----+-----+-----+-----+ 1500

TGTGAAAAGAAAATCGATTACTGCAGTTCAGCCCTTGTGCTAATGGAGCCCAGTGCCTT
1501 -----+-----+-----+-----+-----+-----+ 1560

GACCTGGGGAACCTCTACATATGCCAGTGCCAGGCTGGCTTCACTGGCAGGCACTGTGAC
1561 -----+-----+-----+-----+-----+-----+ 1620

GACAACGTGGACGATTGCGCCTCCTTCCCTGCGTCAATGGAGGGACCTGTCAGGATGGG
1621 -----+-----+-----+-----+-----+-----+ 1680

FIG. 1A2
SUBSTITUTE SHEET (RULE 26)

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GTCAACGACTACTCCTGCACCTGCCCCCGGGATACAACGGGAAGAACTGCAGCACGCCG
1681 -----+-----+-----+-----+-----+-----+ 1740

GTGAGCAGATGCGAGCACAAACCCCTGCCACAATGGGGCCACCTGCCACGAGAGAAGCAAC
1741 -----+-----+-----+-----+-----+-----+ 1800

CGCTACGTGTGCGAGTGCGCTCGGGGCTACGGCGGCCTCAACTGCCAGTTCCTGCTCCCC
1801 -----+-----+-----+-----+-----+-----+ 1860

GAGCCACCTCAGGGGCCGGTCATCGTTGACTTCACCGAGAAGTACACAGAGGGCCAGAAC
1861 -----+-----+-----+-----+-----+-----+ 1920

AGCCAGTTTCCCTGGATCGCAGTGTGCGCCGGGATTATTCTGGTCCTCATGCTGCTGCTG
1921 -----+-----+-----+-----+-----+-----+ 1980

TACCAGTCGGTGACGTCATATCAGAAGAGAAAGATGAGTGCATCATAGCAACTGAGGTG
2401 -----+-----+-----+-----+-----+-----+ 2460

TAAACAGACGTGACGTGGCAAAGCTTATCGATACCGTCATCAAGCTT
2461 -----+-----+-----+-----+-----+-----+ 2508

FIG. 1A3

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1 GAATTGGCACGAGGTTTTTTTTTTTTTTTTTCCCTCTTTCTTTCTTTCTTTCTTTGCCATCCGAAG 69
70 AGCTGTAGCCGCGCGGGCTGCACCTAAGGCGTCGGTAGGGGATAACAGTCAGAGACCTCTCTGA 138
139 AAGCAGGAGACGGGACGGTACCCCTCCGGCTCTGCGGGGCGGTGGGCCCCCTCCGTTCTTTCCCCCTC 207
208 CCCGAGAGACACTTCTTTCCCCCCACGAAGACACAGGGGACGAGCGGTGCCCCCTCCGCC 276
277 ATGGAGGCGGCTTCTGCTGACGCTGCCCTCCTCTCGGCGCTGCTGTCGGCTGCCAGGTGACGGC 345
346 TCCGGGTGTTTCAGCTGAAGCTGAGGAGTTGTCAACAAGAAAGGGCTGCTCAGCAACCGCAACTGC 414
415 TCCGGGGGGGGCGGCCCGAGGCGCGGAGAGTGCAGTGCAGACTTCTTCCGCGTCTGC 483
484 CTGAAGCACTACAGGCCAGGCTCTCCCGAGCGCGCTGCACCTACGGCAGCGCCATACCCCCGTC 552
553 CTGGGCGCCAACTCTTCAGCGTCCCGAGGCGGGGCGCGCACCCGCTTACGCAACCCCATC 621
622 CGTTCCCTTCGGCTTCACTGGCCCGGACCTTCTCGCTCATCATGAGGCTCTGCACACCGACTCC 690
691 CCGACGACCTCACACAGAAACCCCGAGCGCTCATAGCGGCTGGCCACCCAGAGGCACTGGCG 759
760 GTGGCGAGGAGTGTCCAGGACCTGCACAGCAGCGGCGCGCACCGACTCAAGTACTCTATCGCTTT 828
829 XXGTGTATGAGCACTACTACGGGGAAGGCTGCTCTGCTTCTGCCGGCCCGTGACGACCGCTTCGGT 897
898 CACTTCACTGTGGAGAGCGTGGCGAGAAGTCTGCAACCCAGGCTGGAAGGGCCAGTACTGCAC TGAG 966
967 CCGATTGCTTGCCTGGGTGTACGAGCAGCACGGCTTCTCGACAACTGGGGAATGCAAGTGCAGA 1035
1036 GTGGTTGGCAGGGCGGTACTGTGACAGTGCATCCGATACCCAGGCTGCTGCACGGTACCTGTCTAG 1104
1105 CAGCATGGCAGTGCAACTGCCAGGAAGGCTGGGGCGGCTTTCTGCAACCCAGGACCTGAAC TACTGC 1173
1174 ACTCACCACAAGCCATGCAAGAATGGTGCCACATGCACCAACACCGGTACGGGAGCTACACTTGTCT 1242
1243 TGCCGACCTGGGTACACAGGCTCCAGCTGCGAGATTGAAATCAACGAATGTGATGCCAACCTTGCAAG 1311
1312 AATGGTGAAGCTGCACGGATCTCGAGAACAGCTATTCCTGTACCTGCCCGCCAGGCTTCTATGGTAA 1380
1381 AACTGTAGCTGAGTGCATGACTTGTGCTGATGGACCGTCTCAATGGAGGGCGATGCATGACAAC 1449
1450 CCTGATGGTGGATACAGCTGCCGCTGCCACTGGGTATTCTGGGTCAACTGTGAAAAGAAATCGAT 1518
1519 TACTGCAGTCCAGCCCTTGCTAATGGAGCCAGTGGTTGACCTGGGGAACCTCTACATATGCCAG 1587
1588 TGCCAGGCTGGCTTCACTGGCAGGCACTGTGACGACAAGTGGACGATTGGGCTCTTCCCTGCGTC 1656
1657 AATGGAGGGACCTGTCAAGGATGGGTCAACGACTACTCTGCACCTGCCCCCGGGATACAACGGGAAG 1725
1726 AACTGCAGCACGCGGTGAGCAGATGCGAGCAACACCCCTGCCACAATGGGGCCACCTGCCACGAGAGA 1794

FIG. 1B1

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1795 AGCAACCGCTACGTGTGGAGTGGCTCGGGGCTACGGGGCCCTCAACTGCCAGTTCCTGCTCCCGAG 1863
1864 CCACCTCAGGGGCGGTTCATCGTTGACTTACCGAGAAGTACACAGAGGGCCAGAACAGCCAGTTTCCC 1932
1933 TGGATCGCAGTGTGCGCCGGGATTATCTGGTCTCATGCTGCTGCTGGTTGGCCGCCATCGTCGTC 2001
2002 TGGGTACGGCTGAAGGTGCAGAGAGGCACCCAGCCGAGGCTGCAGGAGTGAAACGGAGACCATG 2070
2071 AACACCTGGCGAATGCCAGCGCGAGAAGGACATCTCCATCAGCGTCATCGGTGCCACTCAGATTAA 2139
2140 AACACAAATAAGAAAGTAGACTTTCACAGCGATAACTCCGATAAAAACGGCTACAAAGTTAGATACCCA 2208
2209 TCAGTGGATTACAATTTGGTGCATGAATCAAGAAATGAGGACTCTGTGAAAGAGGAGCATGGCAATGC 2277
2278 GAAGCCAAGTGTGAACGTATGATTCAGAGGCAGAGAGAAAAGCGCAGTACAGTAAAAAGTAGTGAC 2346
2347 ACTTCTGAAGAAAACGGCCAGATTTCAGTATATTCCTCACTTCAAAGGACACAAAGTACCAGTCGGTGAC 2415
2416 GTCATATCAGAAGAGAAAGATGAGTGCATCATAGCAACTGAGGTAGTATCCACCTGGCAGTCGGACA 2484
2485 AGCTTGGTGTGATTCCTCCATCCAGCGAGGTGAGGCGGCCCAACCATCTACCTGCTGCCACAGTC 2553
2554 ATCTGTACCCAATGAAAACCTGGCCACCTTCAGTCTGTGGCACTGCAGACGTTGAAAAACCTTGTG 2622
2623 ATTAACATAAGCTCCAGTGGGGTTACAGGGACAGCAATTTTGCAGGCAAGGTATAACTGTAGTGCA 2691
2692 GTTGTAGCTTACTAACCTTACTGACTCATCTTCGTGTGCTTCTGTCAGAGCCTGTTTTGCTGGCA 2760
2761 TTGAGGTGAAGTCTGACCTCTGCATCCTCATAGTCTCTGCTTCTTTTATTAACTCTTCTGCTC 2829
2830 TCTGCTGTGTTTTCTCTCAACAGGTGTAAACAGACGTCAGCTGGCAAGCTT 2883

FIG. 1B2

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1 MGGRFLLTLA LLSALLCRCQ VDGSGVFELK LQEFVNKKGL LSNRNCCRGG GPGGAGQQQC
61 DCKTFFRVCL KHYQASVSPE PPCTYGSALT PVLGANSFSV PDGAGGADPA FSNPIRFPFG
121 FTWPGTFSLI IEALHTDSPD DLT TENPERL ISRLATQRHL AVGEEWSQDL HSSGRTDLKY
181 SYRFVCDEHY YGEGCSVFCR PRDDRFGHFT CGERGEKVCN PGWKGOYCTE PICLPGCDEQ
241 HGFCDKPGEC KCRVWGQGRY CDECIRYPGC LHGTCQQPWQ CNCQEGWGGL FCNQDLNYCT
301 HHKPCKNGAT CTNTGQGSYT CSCRPGYTGS SCEIEINECD ANPCKNGGSC TDLENSYSCT
361 CPPGFYGNKC ELSAMTCADG PCFNNGRCTD NPDGGYSCRC PLGYSGFNCE KKIDYCSSSP
421 CANGAQCVDL GNSYICQCCA GFTGRHCDDN VDDCASFCV NGGTCQDGVN DYSCTCPPGY
481 NGKNCSTPVS RCEHNPCHNG ATCHERSNRY VCECARGYGG LNCQFLLPEP PQGPVIVDFT
541 EKYTEGQNSQ FPWIAVCAGI ILVLMLLGC AAIVVCVRLK VQKRHHQPEA CRSETETMNN
601 LANCQREKDI SISVIGATQI KNTNKKVDFH SDNSDKNGYK VRYPVDYNL VHELKNEDSV
661 KEEHGKCEAK CETYDSEAE KSAVQLKSSD TSEKRPDSV YTSKDTKYQ SVYVISEEKD
721 ECIIATEV

FIG. 2

C-Delta-1	1	M	G	G	R	F	L	L	T	L	A	-	L	S	A	L	L	C	R	C	Q	V	D	G	S	G	V	F	E	L	K	L	Q	E	F	V	N	K	K	G	L	L	S	N	R	N	C	C	R	G	G	G	P	G	G	A	Q	Q	C	60				
X-Delta-1	1	M	G	Q	R	M	L	T	L	L	-	V	L	S	A	V	L	-	C	Q	I	S	C	S	G	L	F	E	L	R	L	Q	E	F	V	N	K	K	G	L	L	G	N	M	N	C	C	R	P	G	S	L	-	A	S	L	Q	R	C	56				
Delta	1	-	-	M	H	W	I	K	C	L	L	T	A	F	I	C	F	T	V	I	V	Q	V	H	S	S	G	S	F	E	L	R	L	K	Y	F	S	N	D	H	G	R	D	N	E	G	R	C	C	S	G	E	S	D	G	A	T	G	K	C	L	G	59	
C-Delta-1	61	D	C	K	T	F	F	R	V	C	L	K	H	Y	Q	A	S	V	S	P	E	P	P	C	T	Y	G	S	A	I	T	P	V	L	G	A	N	S	F	S	V	P	D	G	A	G	A	D	P	A	F	S	N	P	I	R	F	F	G	F	121			
X-Delta-1	57	E	C	K	T	F	F	R	I	C	L	K	H	Y	Q	S	N	V	S	P	E	P	P	C	T	Y	G	A	V	T	P	V	L	G	T	N	S	F	V	P	E	S	-	S	N	A	D	P	T	F	S	N	P	I	R	F	F	G	F	116				
Delta	60	S	C	K	T	R	F	R	L	C	L	K	H	Y	Q	A	T	I	D	T	S	Q	C	T	Y	G	D	V	I	T	P	I	L	G	E	N	S	V	N	L	T	D	A	Q	R	F	Q	N	K	G	E	T	N	P	I	Q	E	F	F	S	F	120		
C-Delta-1	122	T	W	P	G	T	F	S	L	I	I	E	A	L	H	T	D	S	P	D	L	T	E	N	P	E	R	L	I	S	R	L	A	T	Q	R	H	L	A	V	G	E	E	W	S	Q	D	L	H	S	S	G	R	T	D	L	K	Y	S	Y	182			
X-Delta-1	117	T	W	P	G	T	F	S	L	I	I	E	A	L	H	T	D	S	A	D	D	L	N	T	E	N	P	E	R	L	I	S	R	L	A	T	Q	R	H	L	T	V	G	E	Q	W	S	O	D	L	H	S	S	D	R	T	E	L	K	Y	S	Y	177	
Delta	121	S	W	P	G	T	F	S	L	I	V	E	A	W	H	-	D	T	N	S	G	N	A	R	T	N	K	L	L	I	Q	R	L	V	Q	V	L	E	V	S	S	E	W	K	T	N	K	S	E	S	Q	Y	T	S	L	E	Y	D	F	180				
C-Delta-1	183	R	F	V	C	D	E	H	Y	Y	G	E	G	C	S	V	F	C	R	P	R	D	D	R	F	G	H	F	T	C	G	E	R	G	E	K	V	C	N	P	G	W	K	G	Q	Y	C	T	E	P	I	C	L	P	G	C	D	E	Q	H	G	F	243	
X-Delta-1	178	R	F	V	C	D	E	H	Y	Y	G	E	G	C	S	D	Y	C	R	P	R	D	D	A	F	G	H	F	S	C	G	E	R	G	E	K	L	C	N	P	G	W	K	G	L	Y	C	T	E	P	I	C	L	P	G	C	D	E	H	H	G	Y	238	
Delta	181	R	V	T	-	D	L	N	Y	Y	G	S	G	C	A	K	E	C	R	P	R	D	D	S	E	F	G	H	S	T	C	S	E	T	G	E	I	I	C	L	T	G	W	Q	G	D	Y	C	H	I	P	K	C	A	K	G	C	E	-	-	H	G	H	239
C-Delta-1	244	C	D	K	P	G	E	C	K	C	R	V	G	W	Q	G	R	Y	C	D	E	C	I	R	Y	P	G	C	L	H	G	T	C	Q	Q	P	W	Q	C	N	C	Q	E	G	W	G	G	L	F	C	N	Q	D	L	N	Y	C	T	H	H	K	P	304	
X-Delta-1	239	C	D	K	P	G	E	C	K	C	R	V	G	W	Q	G	R	Y	C	D	E	C	I	R	Y	P	G	C	L	H	G	T	C	Q	Q	P	W	Q	C	N	C	Q	E	G	W	G	L	F	C	N	Q	D	L	N	Y	C	T	H	H	K	P	299		
Delta	240	C	D	K	P	N	Q	C	V	C	Q	L	G	W	K	G	A	L	C	N	E	C	V	L	E	P	N	C	I	H	G	T	C	N	K	P	W	T	C	I	C	N	E	G	W	G	G	L	Y	C	N	O	D	L	N	Y	C	T	N	H	R	P	300	
C-Delta-1	305	C	K	N	G	A	T	C	T	N	T	G	Q	G	S	Y	T	C	S	C	R	P	G	Y	T	G	S	S	C	E	I	E	I	N	E	C	D	A	-	-	N	P	C	K	N	G	G	S	C	T	D	-	-	-	L	E	N	S	Y	S	C	T	360	
X-Delta-1	300	C	E	N	G	A	T	C	T	N	T	G	Q	G	S	Y	T	C	S	C	R	P	G	Y	T	G	S	N	C	E	I	E	V	N	E	C	D	A	-	-	N	P	C	K	N	G	G	S	C	S	D	-	-	-	L	E	N	S	Y	T	C	S	355	
Delta	301	C	K	N	G	G	T	C	F	N	T	G	E	G	L	Y	T	C	K	C	A	P	G	Y	S	G	D	C	E	N	E	I	Y	S	C	D	A	D	V	N	P	C	Q	N	G	G	T	C	I	D	E	P	H	T	K	T	G	Y	K	C	H	361		
C-Delta-1	361	C	P	P	G	F	Y	G	K	N	C	E	L	S	A	M	T	C	A	D	G	P	C	F	N	G	-	-	-	G	R	C	T	D	N	P	D	G	G	Y	S	C	R	C	P	L	G	Y	S	G	F	N	C	E	K	K	I	D	Y	C	416			
X-Delta-1	356	C	P	P	G	F	Y	G	K	N	C	E	L	S	A	M	T	C	A	D	G	P	C	F	N	G	-	-	-	G	R	C	A	D	N	P	D	G	G	Y	I	C	F	C	P	G	V	Y	S	G	F	N	C	E	K	K	I	D	Y	C	411			
Delta	362	C	R	N	G	S	G	K	M	C	E	K	V	L	T	C	S	D	K	P	C	H	Q	G	I	C	R	N	V	R	P	G	L	G	S	K	G	Q	G	Y	Q	C	E	C	P	I	G	Y	S	G	P	N	C	D	L	Q	L	D	N	C	422			

FIG. 3A

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C-Delta-1	184	V	C	D	E	H	Y	G	E	G	C	S	V	F	C	R	P	R	D	D	R	F	G	H	F	T	C	G	E	R	G	E	K	V	C	N	P	G	W	K	G	Q	Y	C	228		
Delta	182	V	T	C	D	L	N	Y	G	S	G	C	A	R	F	C	R	P	R	D	D	S	F	G	H	S	T	C	S	E	T	G	E	I	I	C	L	T	G	W	Q	G	D	Y	C	226	
Serrate	235	V	Q	C	A	V	T	Y	N	T	T	C	T	F	C	R	P	R	D	D	Q	F	G	H	Y	A	C	G	S	E	G	Q	K	L	C	L	N	G	W	Q	G	V	N	C	279		
C-Serrate-1		V	T	C	A	E	H	Y	G	F	G	C	N	K	F	C	R	P	R	D	D	E	F	T	H	T	C	D	Q	N	G	N	K	T	C	L	E	G	W	T	G	P	E	C			
Apex-1	130	N	L	C	S	S	N	Y	H	G	K	R	C	N	R	Y	C	I	A	N	-	A	K	L	H	W	E	-	C	S	T	H	G	V	R	R	C	S	A	G	W	S	G	E	D	C	172
Lag-2	120	V	T	C	A	R	N	Y	F	G	N	R	C	E	N	F	C	D	A	H	L	A	K	A	A	R	K	R	C	D	A	M	G	R	L	R	C	D	I	G	W	M	G	P	H	C	166

FIG. 4

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FIG.5A

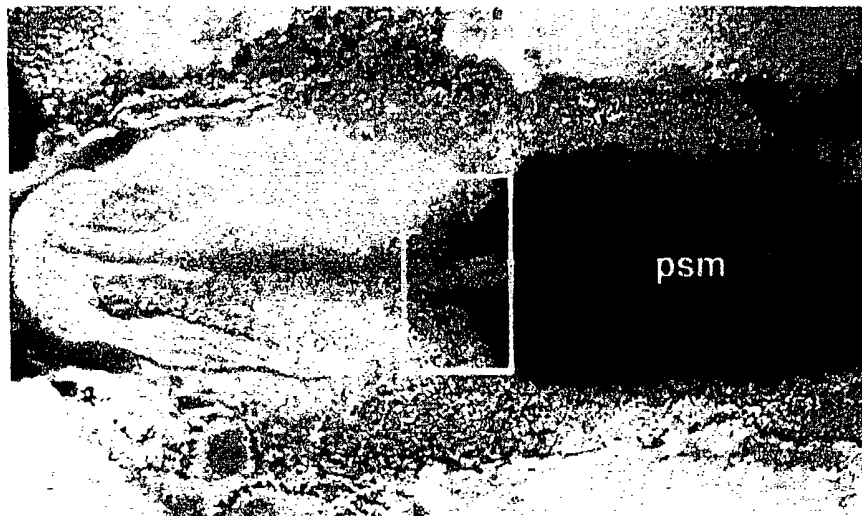


FIG.5B

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FIG.5C



FIG.5D

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FIG.5E

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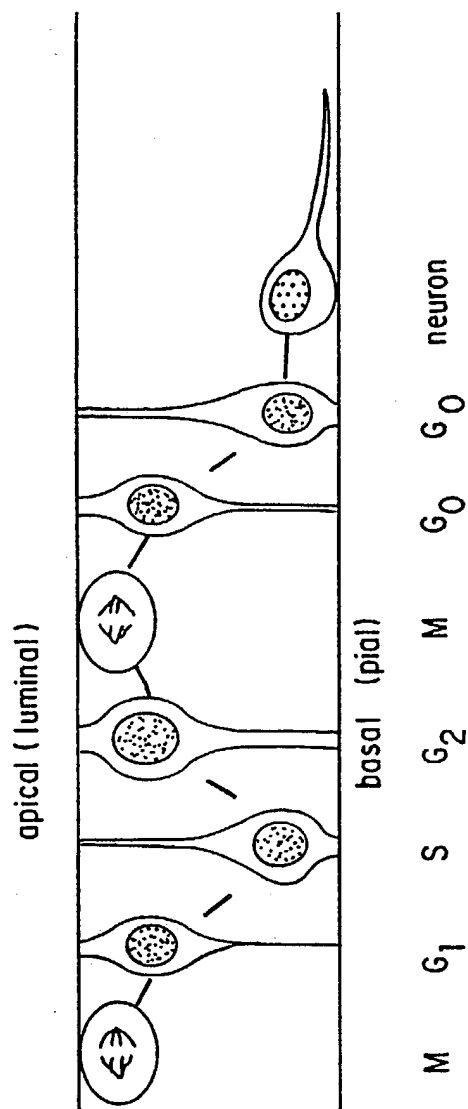


FIG. 6A

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FIG.6B



FIG.6C

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CTGCAGGAAT TCSMYGCGAT GCTCCCGGCC GCCATGGGCC GTCGGAGCGC GTAGCCCTTT 60
GCCGTGGTCT CTGCCCTGCT GTGCCAGGTC TGGAGCTCCG GCGTATTGA GCTGAAGCTG 120
CAGGAGTTCC TCAACAAGAA GGGCTGCTG GGAACCGCA ACTGCTGCCG CGGGGCTCT 180
GGCCCGCCTT GCGCCTGCAG GACCTTCTTT CCGTATGCC TCAAGCACTA CCAGGCCAGC 240
GTGTACACCG AGCCACCCCTG CACCTACGGC AGTGCCGTCA CGCCAGTGCT GGTGTGCGAC 300
TCCTTCAGCC TGCCCTGATG CGCAGGCATC AGGTACCTTC TCTCTGATCA TTGAAGCCCT CATCCGATTC 360
CCCTTCGGCT TCACCTGGCC ACCTCGCAAC AGAAACCCCA GAAAGACTCA TCAGCCGCCCT CCATACAGAC 420
TCTCCCGATG ATCTCGCAAC AGAATGGTCT CAGGACCTTC ACAGTAGCGG GACCACACAG 480
AGGCACCTCA CTGTGGGAGA AGAATGGTCT CAGGACCTTC ACAGTAGCGG CCGCACAGAC 540
CTCCGGTACT CTTACCGGTT TGTGTGTGAC GAGCACTACT ACGGAGAAGG TTGCTCTGTG 600
TTCTGCCGAC CTCGGGATGA CGCCTTTGGC CACTTCACCT GCGGGGACAG AGGGGAGAAG 660
ATGTGCGACC CTGGCTGGAA AGGCCAGTAC TGCACCTGACC CAATCTGTCT GCCAGGTGT 720
GATGACCAAC ATGGATACTG TGACAAACCA GGGAGTGA AGTGCAGAGT TGGCTGGCAG 780
GGCCGCTACT GCGATGAGTG CATCCGATAC CCAGGTTGTC TCCATGGCAC CTGCCAGCAA 840
CCCTGGCAGT GTAACTGCCC GGAAGGCTGG GGGGCCCTTT TCTGCAACCA AGACCTGAAC 900
TACTGTACTC ACCATAAGCC GTGCAGGAAT GGAGCCACCT GCACCAACAC GGGCCAGGGG 960
AGCTACACAT GTTCCCTGCCG ACCTGGGTAT ACAGGTGCCA ACTGTGAGCT GGAAGTAGAT 1020
GAGTGTGCTC CTAGCCCCTG CAAGAACGGA GCGAGCTGCA CGGACCTTGA GGACAGCTTC 1080
TCTTGACACT GCCCTCCCGG CTTCTATGGC AAGGTCTGTG AGCTGAGCGC CATGACCTGT 1140
GCAGATGGCC CTTGCTTCAA TGGAGGACGA TGTTCAGATA ACCCTGACGG AGGCTACACC 1200
TGCCATTGCC CCTTGGGCTT CTCTGGCTTC AACTGTGAGA AGAAGATGGA TCTCTGCGGC 1260
TCTTCCCTTT GTTCTAACGG TGCCAAAGTGT GTGGACCTCG GCAACTCTTA CCTGTGCCGG 1320
TGCCAGGCTG GCTTCTCCGG GAGGTACTGC GAGGACAATG TGGATGACTG TGCCCTCCCTCC 1380

FIG. 7A

SUBSTITUTE SHEET (RULE 28)

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1440 CCGTGTGCAA ATGGGGGCAC CTGCCGGGAC AGTGTGAACG ACTTCTCCTG TACCTGCCCA
1500 CCTGGCTACA CGGGCAAGAA CTGCAGCGCC CCTGTACAGCA GGTGTAGCA TGCACCCCTGC
1560 CATAATGGGG CCACCTGCCA CCAGAGGGGC CAGCGCTACA TGTGTAGTG CGCCAGGGC
1620 TATGGCGGCC CCAACTGCCA GTTCTGCTC CCTGAGCCAC CACCAAGGCC CATGGTGGTG
1680 GACCTCAGTG AGAGGCATAT GGAGAGCCAG GCGGGCCCT TCCCCTGGGT GGCCGTGTGT
1740 GCGGGGGTGG TGCTTGTCTT CCTGCTGCTG CTGGGCTGTG CTGCTGTGGT GGTCTGCCGC
1800 CGGCTGAAGC TACAGAAACA CCAGCCTCCA CCTGAACCTT GTGGGGGAGA GACAGAAACC
1860 ATGAACAACC TAGCCAATTG CCAGCGCGAG AAGGACGTTT CTGTTAGCAT CATGGGGCT
1920 ACCCAGATCA AGAACACCAA CAAGAAGGCG GACTTTCACG GGGACCATGG AGCCGAGAAG
1980 AGCAGCTTTA AGTCCGATA CCCACTGTG GACTATAACC TCGTTCGAGA CCTCAAGGA
2040 GATGAAGCCA CGGTCAGGGA TACACACAGC AAACGTGACA CCAAGTGCCA GTCACAGAGC
2100 TCTGCAGGAG AAGAGAAGAT CGCCCCAACA CTTAGGGGTG GGGAGATTCC TGACAGAAAA
2160 AGGCCAGAGT CTGTCTACTC TACTTCAAAG GACACCAAGT ACCAGTCGGT GTATGTTCTG
2220 TCTGCAGAAA AGGATGAGTG TGTATAGCG ACTGAGGTGT AAGATGGAAG CGATGTGCA
2280 AAATCCCAT TTCTCTTAA TAAATTTCCA AGGATATAGC CCCGATGAAT GCTGCTGAGA
2340 GAGGAAGGA GAGGAAACCC AGGACTGCT GCTGAGAAC CCAGTTCAGG GAACGTGGTT
2400 CTCTCAGAGT TAGCAGAGG GCGCGACACT GCCAGCCTAG GCTTTGGCTG CCGCTGGACT
2460 GCCTGCTGGT TGTTCCTTAT TAGGAAGCAC GCACTGCCCC CAGTGTCTAT TATTTAAATG
2520 GACGAGTGAC TTGATTCTATA TAGGAAGCAC GCACTGCCCC CACGTCTATC TTGGATTACT
2580 ATGAGCCAGT CTTTCCCTTGA ACTAGAAACA CAACTGCCCT TATTGTCCTT TTTGATACTG
2640 AGATGTGTTT TTTTCTTTTC CTAGACGGGA AAAAGAAAC GTGTGTTATT TTTTGTGGA
2692 TTTGTAAAAA TATTTTTCAT GATTATGGGA GAGCTCCCAA CGCGTTGGAG GT

FIG. 7B

SUBSTITUTE SHEET (RULE 28)

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MGRRSALALA VVSALLCQVW SSGVFELKLQ EFVNKKGLLG NRNCCRGSG 50
PPACRRTFFR VCLKHYQASV SPEPPCTYGS AVTPVLGVDS FSLPDGAGID 100
PAFSNPIRFP FGFTWPGTFS LIIEALHTDS PDDLATENPE RLISRLTTQR 150
HLTVGEWSQ DLHSSGRITDL RYSYRFVUDE HYEGECVSF CRPRDDAFGH 200
FTCGDRGEKM CDPGWKGQYC TDPICLPGCD DQHGVCCKPG ECKCRVWQ 250
RYCDECIRYP GCLHGTCCQP WQCNCQEGWG GLFCNQDLNY CTHHKPCRNG 300
ATCTNTGQS YTCSCRPGYT GANCELEVDE CAPSPCKNGA SCTDLEDSEFS 350
CTCPPGFGYK VCELSAMTCA DGPCFNGGRC SDNPDGGYTC HCPLGFSGFN 400
CEKKMDLCGS SPCSNKAKCV DLGNSYLCRC QAGFSGRYCE DNVDDCASSP 450
CANGGTCRDS VNDFSCTCP GYTGNKCSAP VSRCEHAPCH NGATCHQRGQ 500
RYMCECAQGY GGPNCQFLLP EPPPGPMVVD LSEHMHESQG GPFPPWAVCA 550
GVVLVLLLLL GCAAVVVCVR LKLQKHQPPP EPCGGETETM NNLANCQREK 600
DVSVSIIGAT QIKNTNKKAD FHGDHGAES SFKVRYPTVD YNLVRDLKGD 650
EATVRDTHSK RDTKCQSQSS AGEKIAPTL RGGEIPDRKR PESVYSTSKD 700
TKYQSVYVLS AEKDECVIAT EV 722

FIG. 8

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CHICK DELTA	MGGRFLTLA	LLSALLORCO	VDGSGVFELK	LQEFVNKKGL	LSNRNCCRG	50
MOUSE DELTA.PEP	MGRFSALALA	VVSALLCO	VWSSGVFELK	LQEFVNKKGL	LGNRNCCRG	48
CONSENSUS	MG.R...L..LA	...SALLC...	V..SGVFELD	LQEFVNKKGL	L..NRNCCRG	50
CHICK DELTA	GPGGAGQCC	DKITFFRVCL	KHYQASVSPE	PPCTYCSA	IT PVLGANSFS	100
MOUSE DELTA.PEP	—SCP—PC	AKITFFRVCL	KHYQASVSPE	PPCTYCSA	IT PVLGVDSFS	93
CONSENSUS	...G.....C	.C.ITFFRVCL	KHYQASVSPE	PPCTYCSA	.IT PVLG..SFS.	100
CHICK DELTA	PDGAGADPA	FSNPIRFPFG	FTWPGTFSLI	IEALHTDSPD	DLITENPERL	150
MOUSE DELTA.PEP	PDGAG—DPA	FSNPIRFPFG	FTWPGTFSLI	IEALHTDSPD	DLITENPERL	142
CONSENSUS	PDGAG..DPA	FSNPIRFPFG	FTWPGTFSLI	IEALHTDSPD	DL..TENPERL	150
CHICK DELTA	ISRLATORHL	AVGEEWSQDL	HSSGRDLY	SYRFVDEHY	YGEGCSVFCR	200
MOUSE DELTA.PEP	ISRLTATORHL	TVGEEWSQDL	HSSGRDLY	SYRFVDEHY	YGEGCSVFCR	192
CONSENSUS	ISRL..TORHL	..VGEEWSQDL	HSSGRDLY	SYRFVDEHY	YGEGCSVFCR	200
CHICK DELTA	PRDDFGHFT	CGERGEKVCN	PGWKQGYCTE	PICLPGCCDQ	HGCDKPGEC	250
MOUSE DELTA.PEP	PRDDAFGHFT	CGERGEKVCN	PGWKQGYCTD	PICLPGCCDQ	HGCDKPGEC	242
CONSENSUS	PRDD..FGHFT	CG..ERGEK..C	PGWKQGYCT.	PICLPGCCD..Q	HG..CDKPGEC	250
CHICK DELTA	KCRVGWQGRY	CDECIRYPGC	LHGTCCQPWQ	CNCQEGWGGL	FCNQDLNYCT	300
MOUSE DELTA	KCRVGWQGRY	CDECIRYPGC	LHFTCCQPWQ	CNCQEGWGGL	FCNQDLNYCT	292
CONSENSUS	KCRVGWQGRY	CDECIRYPGC	LHGTCCQPWQ	CNCQEGWGGL	FCNQDLNYCT	300
CHICK DELTA	HHKPCNGAT	CTNTGQGSY	CSCRPGYTGS	SCLEINECD	ANPCKNGSC	350
MOUSE DELTA.PEP	HHKPCNGAT	CTNTGQGSY	CSCRPGYTGA	NCELEVDECA	PSPCKNGASC	342
CONSENSUS	HHKPC..NGAT	CTNTGQGSY	CSCRPGYTG..	..CE..E..EC..	..PCKNG..SC	350
CHICK DELTA	TDLENSYST	CPPGFYKMC	ELSAMTCADG	PCFNGGROTD	NPDGGYSORC	400
MOUSE DELTA.PEP	TDLEDSYST	CPPGFYKMC	ELSAMTCADG	PCFNGGROSD	NPDGGYTCHC	392
CONSENSUS	TDLE..S..YST	CPPGFYK..C	ELSAMTCADG	PCFNGGRO..D	NPDGGY..C..C	400
CHICK DELTA	PLGYSGFNCE	KKMDYCSSP	QANGACVDL	GNSYLCQCA	GFTRHCDN	450
MOUSE DELTA.PEP	PLGYSGFNCE	KKMDYCSSP	QANGACVDL	GNSYLCQCA	GFSGRYCEDN	442
CONSENSUS	PLG..SGFNCE	KK..D..C..SSP	Q..NGA..CVDL	GNSY..C..CA	GF..GR..C..DN	450

FIG.9A
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CHICK DELTA	VDDCASFPOV	NGGTCDGVN	DYSCTCPPGY	NGKNCSTPVS	RCEHNPCHNG	500
MOUSE DELTA.PEP	VDDCASSPOA	NGGTCDGVN	DFSTCPPGY	ITGKNCSAPVS	RCEHNPCHNG	492
CONSENSUS	VDDCAS.PC.	NGGTCD.VN.D.	SCTCPPGY	.GKNCS.PVS	RCEH.PCHNG	500
CHICK DELTA	ATCHEISNRY	MCECAGYGG	LNCQFLLEP	PQGRVINDFT	EKYTEGNSQ	550
MOUSE DELTA	ATCHEISGORY	MCECAGYGG	PNCQFLLEP	PPGPVNDLS	ERHMSOGGP	542
CONSENSUS	ATCH.E..RY	.CECA.GYGG	.NCQFLLEP	P.GP..VD..	E....E.Q....	550
CHICK DELTA	FPWIAVCAGI	ILVLM.LLGC	AAIVVCRLK	MOKRHQPEA	CRSETETMNN	600
MOUSE DELTA.PEP	FPWIAVCAGV	VLVLL.LLGC	AAVVCRLK	LKHOPPEP	CGSETETMNN	592
CONSENSUS	FPW.IVCAG.	.LVL.LLGC	AA.VVCRLK	.QK....PE.	C..ETETMNN	600
CHICK DELTA	LANCOREKDI	SISVIGATQI	KNTNKKDFH	SDN-SDKNGY	KVRYPSVDYN	649
MOUSE DELTA	LANCOREKDV	SMSIIGATQI	KNTNKKADFH	GDHGAEKSSF	KVRYPTVDYN	642
CONSENSUS	LANCOREKD.	S.S.IGATQI	KNTNKK.DFH	.D....K...	KVRYP.VDYN	650
CHICK DELTA	LVHELKNEE	SVKEEKKCE	AKCETYDSEA	EEKSAVOLKS	SDTSERKRPF	698
MOUSE DELTA.PEP	LVRDLKDEA	TVRDTHSKRD	TKCOSQSSAG	EEKIAPTLRG	GEIPDRKRFE	692
CONSENSUS	LV..LK....	.M...H.K...	.KC....S.	EEK.A....RKRF.	700
CHICK DELTA	SVYSTSKDTK	YQSVYVISEE	KDECI	IATEV		728
MOUSE DELTA.PEP	SVYSTSKDTK	YQSVYVLSAE	KDECV	IATEV		722
CONSENSUS	SVYSTSKDTK	YQSVYV.S.E	KDEC.	IATEV		730

FIG.9B

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```

      10      20      30      40      50      60
      *      *      *
TACGATGAAY AACCTGGCGA ACTGCCAGCG TCAGAAGGAC ATCTCAGTCA GCATCATCGG
Y D E X P G E L P A * E G H L S Q H H R>
T M N N L A N C Q R E K D I S V S I I G>
R * X T W R T A S V R R T S Q S A S S>

      70      80      90      100     110     120
      *      *      *
GGCYACGTCA GATCARGAAC ACCAACAAGA AGGCGGACTT YMCASCGGGG GACCASAGCG
G X V R S X T P T R R R T X X R G T X A>
A T S D Q E H Q Q E G G L X X G G P X R>
G X R Q I X N T N K K A D F X X G D X S>

      130     140     150     160     170     180
      *      *      *
TCCGACAAGA ATGGMTTTC AAGCCYGCTA CCCCAGCGTG GACTATAACT CGTGCAGGAC
S D K N G F Q G P L P Q R G L * L V Q D>
P T R M X F K A R Y P S V D Y N S C R T>
V R Q E W X S R P A T P A W T I T R A G>

      190     200     210     220     230     240
      *      *      *
CTCAAGGGTG ACGACACCGC CGTCAGGACG TCGCACAGCA AGCGTGACAC CAAGTGCCAG
L K G D D T A V R T S H S K R D T K C Q>
S R V T T P P S G R R T A S V T P S A S>
P Q G * R H R R Q D V A Q Q A * H Q V P>

      250     260     270     280     290     300
      *      *      *
TCCCCAGGCT CCTCAGGGAG GAGAAGGGGA CCCCAGCAC ACTCAGGGGK TCGTGCTGC
S P G S S G R R R G P R P H S G X A C C>
P Q A P Q G G E G D P D H T Q G X R A A>
V P R L L R E E K G T P T T L R G C V L>

      310     320     330     340     350     360
      *      *      *
GGGCCGGGCT CAGGAGGGGG TACCTGGGGG GTGTCTTCCT GGAACCACTG CTCCGTTTCT
G P G S G G G T W G V S S W N H C S V S>
G R A Q E G V P G G C L P G T T A P F L>
R A G L R R G Y L G G V F L E P L L R F>

```

FIG. 10A

SUBSTITUTE SHEET (RULE 26)

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```
      370      380      390      400      410      420
              *              *              *
CTTCCCAAAT GTTCTCATGC ATTCATTGTG GATTTTCTCT ATTTTCCTTT TAGTGGAGAA
L P K C S H A F I V D F L Y F P F S G E>
F P N V L M H S L W I F S I F L L V E K>
S S Q M F S C I H C G F S L F S F * W R>

      430      440      450      460      470      480
              *              *              *
GCATCTGAAA GAAAAAGGCC GGACTCGGGC TGTTCAACTT CAAAAGACAC CAAGTACCAG
A S E R K R P D S G C S T S K D T K Y Q>
H L K E K G R T R A V Q L Q K T P S T S>
S I * K K K A G L G L F N F K R H Q V P>

      490      500      510      520
              *              *
TCGGTGTACG TCATATCCGA GGAGAAGGAC GAGTGCGTCA TCGCA
S V Y V I S E E K D E C V I A>
R C T S Y P R R R T S A S S>
V G V R H I R G E G R V R H R>
```

FIG. 10B

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```

1  TMNNLANCQREKDISVSIIGATQIXNTNKKADFXXGDSSDKNGFQKARY  50
   |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
597 TMNNLANCQREKDISISVIGATQIKNTNKKVDFHSDN..SDKNGY.KVRY 643

51  PSVDYNLVQDLKGDDTAVRTSHSKRDTKCQSPGSSGRRRGPRPHSGXACC 100
   |||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
644 PSVDYNLVHELKNE.DSVKEEHGKCEAKCETYDSEAEKSA..... 683

101 GPGSGGGTGWGVSSWNHCSVSLPKCSHAFIVDFLYFPFSGEASERKRPDSG 150
   |||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
684 .....VQLK....SSDTSERKRPDSV 700

151 CSTSKDTKYQSVYVISEEKDECIVIA 175
   :|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:
701 YSTSKDTKYQSVYVISEEKDECIIA 725

```

FIG.11

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10	20	30	40	50	60
* *	* *	* *	* *	* *	* *
CATTGGGTAC	GGGCCCCCCT	CGAGGTCGAC	GGTATCGATA	AGCTTGATAT	CGAATTCCGG
70	80	90	100	110	120
* *	* *	* *	* *	* *	* *
CTTCACCTGG	CCGGGCACCT	TCTCTCTGAT	TATTGAAGCT	CTCCACACAG	ATTCTCCTGA
130	140	150	160	170	180
* *	* *	* *	* *	* *	* *
TGACCTCGCA	ACAGAAAACC	CAGAAAGACT	CATCAGCCGC	CTGGCCACCC	AGAGGCACCT
190	200	210	220	230	240
* *	* *	* *	* *	* *	* *
GACGGTGGGC	GAGGAGTGGT	CCCAGGACCT	GCACAGCAGC	GGCCGCACGG	ACCTCAAGTA
250	260	270	280	290	300
* *	* *	* *	* *	* *	* *
CTCCTACCGC	TTCGTGTGTC	ACCAACACTA	CTACGGAGAG	GGCTGCTCCG	TTTCTGCCG
310	320	330	340	350	360
* *	* *	* *	* *	* *	* *
TCCCCGGGAC	GATGCCTTCG	GCCACTTCAC	CTGTGGGGAG	CGTGGGGAGA	AAGTGTGCAA
370	380	390	400	410	420
* *	* *	* *	* *	* *	* *
CCCTGGCTCG	AAAGGGCCCT	ACTGCACAGA	GCCGATCTGC	CTGCCTGGAT	GTGATGAGCA
430	440	450	460	470	480
* *	* *	* *	* *	* *	* *
GCATGGATTT	TGTGACAAAC	CAGGGGAATG	CAAGTGCAGA	GTGGGCTGGC	AGGGCCGGTA
490	500	510	520	530	540
* *	* *	* *	* *	* *	* *
GTGTGACGAG	TGTATCCGCT	ATCCAGGCTG	TCTCCATGGC	ACCTGCCAGC	AGCCCTGGCA
550	560	570	580	590	600
* *	* *	* *	* *	* *	* *
GTGCAACTGC	CAGGAAGGNT	GGGGGGGCCT	TTTCTGCAAC	CAGGACCTGA	ACTACTGCAC
610	620	630	640	650	660
* *	* *	* *	* *	* *	* *
ACACCATAAG	CCCTGCAAGA	ATGGAGCCAC	CTGCAACAAA	CACGGGCCAG	GGGGAGCTAC
670	680	690	700	710	720
* *	* *	* *	* *	* *	* *
ACTTGGTCTT	TGGCCGGNCT	GGGGTACANA	GGGTGCCACC	TGCGAAGCTT	GGGGATTGGA
730	740	750	760	770	780
* *	* *	* *	* *	* *	* *
CGAGTTGTTG	ACCCAGCCCC	TTGGTAAGAA	CGGAGGGAGC	TTGACGGATC	TTCGGAGAAC
790	800	810	820	830	840
* *	* *	* *	* *	* *	* *
AGCTACTCCT	GTACCTGCCC	ACCCGGCTTC	TACGGCAAAA	TCTGTGAATT	GAGTGCCATG
850	860	870	880	890	900
* *	* *	* *	* *	* *	* *
ACCTGTGCGG	ACGGCCCTTG	CTTTAACGGG	GGTCGGTGCT	CAGACAGCCC	CGATGGAGGG

FIG. 12A1
SUBSTITUTE SHEET (RULE 28)

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910	920	930	940	950	960
* *	* *	* *	* *	* *	* *
TACAGCTGCC	GCTGCCCCGT	GGGCTACTCC	GGCTTCAACT	GTGAGAAGAA	AATTGACTAC
970	980	990	1000	1010	1020
* *	* *	* *	* *	* *	* *
TGCAGCTCTT	CACCCTGTTC	TAATGGTGCC	AAGTGTGTGG	ACCTCGGTGA	TGCCTACCTG
1030	1040	1050	1060	1070	1080
* *	* *	* *	* *	* *	* *
TGCCGCTGCC	AGGCCGGCTT	CTCGGGGAGG	CACTGTGACG	ACAACGTGGA	CGACTGCGCC
1090	1100	1110	1120	1130	1140
* *	* *	* *	* *	* *	* *
TCCTCCCCGT	GCGCCAACGG	ACCTCGGTGA	CGGGATGGCG	TGAACGACTT	CTCCTGCACC
1150	1160	1170	1180	1190	1200
* *	* *	* *	* *	* *	* *
TGCCCCGCTG	GCTACACGGG	CAGGAAGTGC	AGTGCCCCCG	CCAGCACCTG	CGAGCACGCA
1210	1220	1230	1240	1250	1260
* *	* *	* *	* *	* *	* *
CCCTGCCACA	ATGGGGCCAC	CTGCCACGAG	AGGGGCCACC	GCTATNTGTG	CGAGCACGCA
1270	1280	1290	1300	1310	1320
* *	* *	* *	* *	* *	* *
CGAAGTACG	GGGGTCCCAA	CTCCANTTC	CTGCTCCCC	AAACTGCCCC	CCCGGCCCCA
1330	1340	1350	1360	1370	1380
* *	* *	* *	* *	* *	* *
CGGTGGTGA	AACTCCCCTA	AAAAAACCTA	AAAGGGCCGG	GGGGGGCCCA	TCCCCTTGTT
1390	1400	1410	1420	1430	1440
* *	* *	* *	* *	* *	* *
GGACGTGTGC	GCCGGGGTCA	TCCTTGTCTT	CATGCTGCTG	CTGGGCTGTG	CCGCTGTGGT
1450	1460	1470	1480	1490	1500
* *	* *	* *	* *	* *	* *
GGTCTGCGTC	CGGCTGAGGC	TGCAGAAGCA	CCGGCCCCCA	GCCGACCCCT	GNCGGGGGGA
1510	1520	1530	1540	1550	1560
* *	* *	* *	* *	* *	* *
GACGGAGACC	ATGAACAACC	TGGNCAACTG	CCAGCGTGAG	AAGGACATCT	CAGTCAGCAT
1570	1580	1590	1600	1610	1620
* *	* *	* *	* *	* *	* *
CATCGGGGNC	ACGCAGATCA	AGAACACCAA	CAAGAAGGCG	GACTTCCACG	GGGACCACAG
1630	1640	1650	1660	1670	1680
* *	* *	* *	* *	* *	* *
NGCCGACAAG	AATGGCTTCA	AGGCCCGCTA	CCCAGNGGTG	GACTATAACC	TCGTGCAGGA
1690	1700	1710	1720	1730	1740
* *	* *	* *	* *	* *	* *
CCTCAAGGGT	GACGACACCG	CCGTGAGCCA	CGCGCACAGC	AAGCGTGACA	CCAAGTGNCA
1750	1760	1770	1780	1790	1800
* *	* *	* *	* *	* *	* *
GCCCCAGGGC	TCCTCAGGGG	AGGAGAAGGG	GACCCCCGAC	CCACACTCAG	GGGGTGGAGG

FIG. 12A2
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1810	1820	1830	1840	1850	1860
* *	* *	* *	* *	* *	* *
AAGCATCTTG	AAAGAAAAAG	GCCGGACTTC	GGGCTTGTTT	AACTTTCAAA	AGACAANCAA
1870	1880	1890	1900	1910	1920
* *	* *	* *	* *	* *	* *
NGTACAAGTC	GGTGTNCGTC	ATTTCGNAG	GAGGAAGGNT	GA CTGCGTCA	TAGGAANTTG
1930	1940	1950	1960	1970	1980
* *	* *	* *	* *	* *	* *
AGGTNGTAAA	NTGGNAGTTG	ANNTTGAAA	GNNNTCCCCG	GATTCCGNTT	TCAAAGTTTT

T

FIG. 12A3

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10 20 30 40 50 60
 * * * * * * * * * * * * * * * * a.a.no.
 CATTGGGTAC GGGCCCCCCT CGAGGTCGAC GGTATCGATA AGCTTGATAT CGAATTCCTGG
 H W V R A P L E V D G I D K L D I E F R> 20
 I G Y G P P S R S T V S I S L I S N S G> 20
 L G T G P P R G R R Y R * A * Y R I P> 19

70 80 90 100 110 120
 * * * * * * * * * * * * * * * *
 CTTACCTGG CCGGGCACCT TCTCTGTGAT TATTGAAGCT CTCCACACAG ATTCTCCTGA
 L H L A G H L L S D Y * S S P H R F S *> 40
 F T W P G T F S L I I E A L H T D S P D> 40
 A S P G R A P S L * L L K L S T Q I L L> 39

130 140 150 160 170 180
 * * * * * * * * * * * * * * * *
 TGACCTCGCA ACAGAAAACC CAGAAAGACT CATCAGCCGC CTGGCCACCC AGAGGCACCT
 * P R N R K P R K T H Q P P G H P E A P> 60
 D L A T E N P E R L I S R L A T Q R H L> 60
 M T S Q Q K T Q K D S S A A W P P R G T> 59

190 200 210 220 230 240
 * * * * * * * * * * * * * * * *
 GACCGTGGGC GAGGAGTGGT CCCAGGACCT GCACAGCAGC GGCCGCACGG ACCTCAAGTA
 D G G R G V V P G P A Q Q R P H G P Q V> 80
 T V G E E W S Q D L H S S G R T D L K Y> 80
 * R W A R S G P R T C T A A A A R T S S> 79

250 260 270 280 290 300
 * * * * * * * * * * * * * * * *
 CTCCTACCGC TTCGTGTGTG ACGAACAATA CTACGGAGAG GGCTGCTCCG TTTTCTGCCG
 L L P L R V * R T L L R R G L L R F L P> 100
 S Y R F V C D E H Y Y G E G C S V F C R> 100
 T P T A S C V T N T T T E R A A P F S A> 99

310 320 330 340 350 360
 * * * * * * * * * * * * * * * *
 TCCCCGGGAC GATGCCTTCG GCCACTTCAC CTGTGGGGAG CGTGGGGAGA AAGTGTGCAA
 S P G R C L R P L H L W G A W G E S V Q> 120
 P R D D A F G H F T C G E R G E K V C N> 120
 V P G T M P S A T S P V C S V G R K C A> 119

FIG.12B1
 SUBSTITUTE SHEET (RULE 26)

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| | | | | | |
|--|-----------|-----------|-----------|-----------|-----------|
| 370 | 380 | 390 | 400 | 410 | 420 |
| * * * * * | * * * * * | * * * * * | * * * * * | * * * * * | * * * * * |
| CCCTGGCTGG AAAGGGCCCT ACTGCACAGA GCCGATCTGC CTGCCCTGGAT GTGATGAGCA | | | | | |
| P W L E R A L L H R A D L P A W M * * A> 140 | | | | | |
| P G W K G P Y C T E P I C L P G C D E Q> 140 | | | | | |
| T L A G K G P T A Q S R S A C L D V M S> 139 | | | | | |
| | | | | | |
| 430 | 440 | 450 | 460 | 470 | 480 |
| * * * * * | * * * * * | * * * * * | * * * * * | * * * * * | * * * * * |
| GCATGGATTT TGTGACAAAC CAGCCCAATG CAAGTGCAGA GTGGGCTGGC AGGGCCCGTA | | | | | |
| A W I L * Q T R G M Q V Q S G L A G P V> 160 | | | | | |
| H G F C D K P G E C K C R V G W Q G R Y> 160 | | | | | |
| S M D F V T N Q G N A S A E W A G R A G> 159 | | | | | |
| | | | | | |
| 490 | 500 | 510 | 520 | 530 | 540 |
| * * * * * | * * * * * | * * * * * | * * * * * | * * * * * | * * * * * |
| CTGTGACGAG TGTATCCGCT ATCCAGGCTG TCTCCATGCC ACCTGCCAGC AGCCCTGGCA | | | | | |
| L * R V Y P L S R L S P W H L P A A L A> 180 | | | | | |
| C D E C I R Y P G C L H G T C Q Q P W Q> 180 | | | | | |
| T V T S V S A I Q A V S M A P A S S P G> 179 | | | | | |
| | | | | | |
| 550 | 560 | 570 | 580 | 590 | 600 |
| * * * * * | * * * * * | * * * * * | * * * * * | * * * * * | * * * * * |
| GTGCAACTGC CAGGAAGGNT GGGGGGGCCT TTTCTGCAAC CAGGACCTGA ACTACTGCAC | | | | | |
| V Q L P G R X G G P F L Q P G P E L L H> 200 | | | | | |
| C N C Q E G W G G L F C N Q D L N Y C T> 200 | | | | | |
| S A T A R K X G G A F S A T R T * T T A> 199 | | | | | |
| | | | | | |
| 610 | 620 | 630 | 640 | 650 | 660 |
| * * * * * | * * * * * | * * * * * | * * * * * | * * * * * | * * * * * |
| ACACCATAAG CCCTGCAAGA ATCGAGCCAC CTGCAACAAA CACGGGCCAG GGGGAGCTAC | | | | | |
| T P * A L Q E W S H L Q Q T R A R G S Y> 220 | | | | | |
| H H K P C K N G A T C N K H G P G G A T> 220 | | | | | |
| H T I S P A R M E P P A T N T G Q G E L> 219 | | | | | |
| | | | | | |
| 670 | 680 | 690 | 700 | 710 | 720 |
| * * * * * | * * * * * | * * * * * | * * * * * | * * * * * | * * * * * |
| ACTTGGTCTT TGCCCGNCT GGGGTACANA GGGTGCCACC TGCGAAGCTT GGGGATTGGA | | | | | |
| T W S L A G L G Y X G C H L R S L G I G> 240 | | | | | |
| L G L W P X W G T X G A T C E A W G L D> 240 | | | | | |
| H L V F G R X C V X R V P P A K L G D W> 239 | | | | | |

FIG.12B2
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| | | | | | |
|---|---------|---------|-----------|-----------|---------|
| 730 | 740 | 750 | 760 | 770 | 780 |
| * * * * | * * * * | * * * * | * * * * | * * * * | * * * * |
| CGAGTTGTG ACCCCAGCCC TTGTAAGAA CCGAGGGAGC TTGACGGATC TTCGGAGAAC | | | | | |
| R V V D | P S P | W | * E R R E | L D G S S | E N> |
| 260 | | | | | |
| E L L T P A L G K N G G S L T D L R R T> | | | | | |
| 260 | | | | | |
| T S C * P Q P L V R T E Q A * R I F G E> | | | | | |
| 259 | | | | | |
| | | | | | |
| 790 | 800 | 810 | 820 | 830 | 840 |
| * * * * | * * * * | * * * * | * * * * | * * * * | * * * * |
| AGCTACTCT GTACCTGCCC ACCCGGCTTC TACGGCAAAA TCTGTGAATT GAGTGGCATG | | | | | |
| S Y S C T C P | P G F | Y G K | I C E L | S A M> | |
| 280 | | | | | |
| A T P V P A H P A S T A K S V N * V P > | | | | | |
| 280 | | | | | |
| Q L L L Y L P T R L L R Q N L * I E C H> | | | | | |
| 279 | | | | | |
| | | | | | |
| 850 | 860 | 870 | 880 | 890 | 900 |
| * * * * | * * * * | * * * * | * * * * | * * * * | * * * * |
| ACCTGTGCGG ACGGCCCTTG CTTTAACGGG GGTGGTGCT CAGACAGCCC CGATGGAGGG | | | | | |
| T C A D G P C | F N G | G R C | S D S P | D G G> | |
| 300 | | | | | |
| P V R T A L A L T G V G A Q T A P M E G> | | | | | |
| 300 | | | | | |
| D L C G R P L L * R G S V L R Q P R W R> | | | | | |
| 299 | | | | | |
| | | | | | |
| 910 | 920 | 930 | 940 | 950 | 960 |
| * * * * | * * * * | * * * * | * * * * | * * * * | * * * * |
| TACAGTGGC GCTGCCCCG CCGCTACTCC GCCTTCAACT GTGAGAAGAA AATTGACTAC | | | | | |
| Y S C R C P V | G Y S | G F N | C E K K | I D Y> | |
| 320 | | | | | |
| T A A A A P W A T P A S T V R R K L T T> | | | | | |
| 320 | | | | | |
| V Q L P L P R G L L R L Q L * E E N * L> | | | | | |
| 319 | | | | | |
| | | | | | |
| 970 | 980 | 990 | 1000 | 1010 | 1020 |
| * * * * | * * * * | * * * * | * * * * | * * * * | * * * * |
| TGCAGCTCT CACCTGTTC TAATGGTGCC AAGTGTGTGG ACCTCGGTGA TGCCTACCTG | | | | | |
| C S S S P C S | N G A | K C V | D L G D | A Y L> | |
| 340 | | | | | |
| A A L H P V L M V P S V W T S V M P T C> | | | | | |
| 340 | | | | | |
| L Q L F T L F * W C Q V C G P R * C L P> | | | | | |
| 339 | | | | | |
| | | | | | |
| 1030 | 1040 | 1050 | 1060 | 1070 | 1080 |
| * * * * | * * * * | * * * * | * * * * | * * * * | * * * * |
| TGCCGCTGCC AGGCCGGCTT CTCGGGGAGG CACTGTGACG ACAACGTGGA CGACTGCGCC | | | | | |
| C R C Q A G F | S G R | H C D | D N V D | D C A> | |
| 360 | | | | | |
| A A A R P A S R G G T V T T T W T T A P> | | | | | |
| 360 | | | | | |
| V P L P G R L L G E A L * R Q R G R L R> | | | | | |
| 359 | | | | | |

FIG.12B3
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| | | | | | | |
|---|------|------|------|------|------|-----|
| 1090 | 1100 | 1110 | 1120 | 1130 | 1140 | |
| * * * * * | | | | | | |
| TCCTCCCCGT GCCCAACGG GGGCACCCTGC CGGGATGGCG TGAACGACTT CTCCTGCACC | | | | | | |
| S S P C A N G G T C R D G V N D F S C T> | | | | | | 380 |
| P P R A P T G A P A G M A * T T S P A P> | | | | | | 380 |
| L L P V R Q R G H L P G W R E R L L L H> | | | | | | 379 |
| | | | | | | |
| 1150 | 1160 | 1170 | 1180 | 1190 | 1200 | |
| * * * * * | | | | | | |
| TCCCCCCTG GCTACACGGG CAGGAACCTGC AGTGCCCCCG CCAGCAGGTG CGAGCAGCA | | | | | | |
| C P P G Y T G R N C S A P A S R C E H A> | | | | | | 400 |
| A R L A T R A G T A V P P P A G A S T H> | | | | | | 400 |
| L P A W L H G Q E L Q C P R Q Q V R A R> | | | | | | 399 |
| | | | | | | |
| 1210 | 1220 | 1230 | 1240 | 1250 | 1260 | |
| * * * * * | | | | | | |
| CCCTGCCACA ATGGGGCCAC CTGCCACGAG AGGGGCCACC GCTATNTGTG CGAGTGTGCC | | | | | | |
| P C H N G A T C H E R G H R Y X C E C A> | | | | | | 420 |
| P A T M G P P A T R G A T A I C A S V P> | | | | | | 420 |
| T L P Q W G H L P R E G P P L F V R V C> | | | | | | 419 |
| | | | | | | |
| 1270 | 1280 | 1290 | 1300 | 1310 | 1320 | |
| * * * * * | | | | | | |
| CGAAGCTACG GGGGTCCCAA CTGCCANTTC CTGCTCCCCG AAAGTGGCCC CCCGGCCCCA | | | | | | |
| R S Y G G P N C X F L L P E T A P P A P> | | | | | | 440 |
| E A T G V P T A X S C S P K L P P R P H> | | | | | | 440 |
| P K L R G S Q L P X P A P R N C P P G P> | | | | | | 439 |
| | | | | | | |
| 1330 | 1340 | 1350 | 1360 | 1370 | 1380 | |
| * * * * * | | | | | | |
| CGGTGCTGGA AACTCCCCTA AAAAAACCTA AAAGGGCCGG GGGGGGCCCA TCCCCTTGCT | | | | | | |
| R W W K L P * K N L K G P G G A H P L G> | | | | | | 460 |
| G G G N S P K K T * K G R G G P I P L V> | | | | | | 460 |
| T V V E T P L K K P K R A G G G P S P W> | | | | | | 459 |
| | | | | | | |
| 1390 | 1400 | 1410 | 1420 | 1430 | 1440 | |
| * * * * * | | | | | | |
| GGAGCTGTGC GCCGGGTCA TCCTTGCTCT CATGCTGCTG CTGGGCTGTC CCGCTGTGCT | | | | | | |
| G R V R R G H P C P H A A A G L C R C G> | | | | | | 480 |
| D V C A G V I L V L M L L L G C A A V V> | | | | | | 480 |
| W T C A P G S S L S S C C C W A V P L W> | | | | | | 479 |

FIG.12B4
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1450 1460 1470 1480 1490 1500
* * * * *
GGTCTGCGTC CGGCTGAGGC TGCAGAAGCA CCGGCCCCCA GCCGACCCCT GNCGGGGGA
G L R P A E A A E A P A P S R P L X G C> 500
V C V R L R L Q K H R P P A D P X R G E> 500
W S A S G * G C R S T G P Q P T P X G G> 499

1510 1520 1530 1540 1550 1560
* * * * *
GACCGAGACC ATGAACAACC TGCNCACTG CCAGCGTGAG AAGGACATCT CAGTCAGCAT
D C D H E Q P G Q L P A * E G H L S Q H> 520
T E T M N N L X N C Q R E K D I S V S I> 520
R R R P * T T W X T A S V R R T S Q S A> 519

1570 1580 1590 1600 1610 1620
* * * * *
CATCGGGGNC ACCGAGATCA AGAACACCAA CAAGAAGGCG GACTTCCACG GGGACCACAG
H R G H A D Q E H Q Q E G G L P R G P Q> 540
I G X T Q I K N T N K K A D F H G D H X> 540
S S G X R R S R T P T R R R T S T G T T> 539

1630 1640 1650 1660 1670 1680
* * * * *
NGCCGACAAG AATGGCTTCA AGCCCGCTA CCCAGNGGTG GACTATAACC TCGTCAGGA
X R Q E W L Q G P L P X G G L * P R A G> 560
A D K N G F K A R Y P X V D Y N L V Q D> 560
X P T R M A S R P A T Q X W T I T S C R> 559

1690 1700 1710 1720 1730 1740
* * * * *
CCTCAAGGT GACGACACCG CCGTCAGGA CGCGCACAGC AAGCGTGACA CCAAGTGCA
P Q G * R H R R Q G R A Q Q A * H Q V X> 580
L K G D D T A V R D A H S K R D T K X Q> 580
T S R V T T P P S G T R T A S V T P S X> 579

1750 1760 1770 1780 1790 1800
* * * * *
GCCCCAGGG TCCTCAGGG AGGAGAAGGG GACCCCGAC CCACACTCAG GGGGTGGAGG
A P G L L R G G E G D P R P T L R I G W R> 600
P Q G S S G E E K G T P D P H S G G G G> 600
S P R A P Q G R R R G P P T H T Q G V E> 599

FIG.12B5
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1810 1820 1830 1840 1850 1860
* * * * *
AAGCATCTTG AAAGAAAAAG GCCGGACTTC GGCCTTGTC AACTTTCAAA AGACAANCAA
K H L E R K R P D F G L V Q L S K D X Q> 620
S I L K E K G R T S G L F N F Q K T X X> 620
E A S * K K K A G L R A C S T F K R Q X> 619

1870 1880 1890 1900 1910 1920
* * * * *
NGTACAAGTC GGTGTNCGTC ATTCCGNAG GAGGAAGNT GACTGCGTCA TAGGAANTG
X T S R C X S F P X E E G * L R H R X L> 640
V Q V G V R H F R R R K X D C V I G X*> 640
X Y K S V X V I S X G G R X T A S * E X> 639

1930 1940 1950 1960 1970 1980
* * * * *
AGGTNGTAAA NTGCNAGTTG ANNTTGGAAA GNNNTCCCCC GATTCCCNNT TCAAAGTTT
R X * X G S * X W K X X P G F R F Q S F> 660
G X K X X V X X G K X S P D S X F K V F> 660
E V V X W X L X L E X X P R I P X S K F> 659

FIG.12B6

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| | | |
|-----------------|---|-----|
| MOUSE DELTA DNA | GTCCAGCGGT ACCATGGGCC GTCGGAGCGC GCTAGCCCTT CCCGTGGTCT | 50 |
| HUMAN DELTA | ----- | |
| CONSENSUS | GTCCAGCGGT ACCATGGGCC GTCGGAGCGC GCTAGCCCTT CCCGTGGTCT | 50 |
| MOUSE DELTA DNA | CTGCCCTGCT GTGCCAGGTC TGGAGCTCCG GCGTATTGA GCTGAAGCTG | 100 |
| HUMAN DELTA | ----- | |
| CONSENSUS | CTGCCCTGCT GTGCCAGGTC TGGAGCTCCG GCGTATTGA GCTGAAGCTG | 100 |
| MOUSE DELTA DNA | CAGGAGTTCC TCAACAAGAA GGGGCTGCTG GGAACCGCA ACTGCTGCCG | 150 |
| HUMAN DELTA | ----- | |
| CONSENSUS | CAGGAGTTCC TCAACAAGAA GGGGCTGCTG GGAACCGCA ACTGCTGCCG | 150 |
| MOUSE DELTA DNA | CGGGGGCTCT GGCCCGCCTT GCGCCTGCAG GACCTTCTTT CCGGTATGCC | 200 |
| HUMAN DELTA | ----- | |
| CONSENSUS | CGGGGGCTCT GGCCCGCCTT GCGCCTGCAG GACCTTCTTT CCGGTATGCC | 200 |
| MOUSE DELTA DNA | TCAAGCACTA CCAGGCCAGC GTGTCACCGG AGCCACCCTG CACCTACGGC | 250 |
| HUMAN DELTA | ----- | |
| CONSENSUS | TCAAGCACTA CCAGGCCAGC GTGTCACCGG AGCCACCCTG CACCTACGGC | 250 |
| MOUSE DELTA DNA | AGTGCTGTCA CGCCAGTGCT GGGTGTGAC TCCTTCAGCC TGCCTGATCG | 300 |
| HUMAN DELTA | -----CATTCG | 5 |
| CONSENSUS | AGTGCTGTCA CGCCAGTGCT GGGTGTGAC TCCTTCAGCC TGCCTSATCG | 300 |
| MOUSE DELTA DNA | CGCAGGCATC GACCCG--G COTTTAGCAA CCCCA--TCC GAT-TC-CCC | 343 |
| HUMAN DELTA | GGTACGGGCC CCCCTCGAGG TCGACGGTAT CGATAAGCTT GATATCGAAT | 55 |
| CONSENSUS | SCYASGSRYC SMCCYCGAGG YCKWCRGYAW CSMYAAGYYY GATATCGMY | 350 |
| MOUSE DELTA DNA | TTCCGGCTTCA CCTGGCCAGG TACCTTCTCT CTGATCATTG AAGCCCTCCA | 393 |
| HUMAN DELTA | TCCGGCTTCA CCTGGCCCGG CACCTTCTCT CTGATTATTG AAGCTCTCCA | 105 |
| CONSENSUS | TTCCGGCTTCA CCTGGCCCGG TACCTTCTCT CTGATYATTG AAGCTCTCCA | 400 |
| MOUSE DELTA DNA | TACAGACTCT CCGATGACC TCGCAACAGA AAACCCAGAA AGACTCATCA | 443 |
| HUMAN DELTA | CACAGATTCT CCGATGACC TCGCAACAGA AAACCCAGAA AGACTCATCA | 155 |
| CONSENSUS | YACAGATTCT CCGATGACC TCGCAACAGA AAACCCAGAA AGACTCATCA | 450 |

FIG. 13A
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| | | |
|-----------------|--|-----|
| MOUSE DELTA DNA | GCCGCCTGAC CACACAGAG CACCTCACTG TGGGAGAAGA ATGGTCTCAG | 493 |
| HUMAN DELTA | GCCGCCTGCC CACCCAGAG CACCTCACC GGGCCGAGGA GTGGTCCCAG | 205 |
| CONSENSUS | GCCGCCTGRC CACACAGAG CACCTCACK TGGGAGAGA RTGGTCTCAC | 500 |
| MOUSE DELTA DNA | GACCTTCACA GTAGCGGCCG CACGACCTC CGGTACTCTT ACCGCTTTGT | 543 |
| HUMAN DELTA | GACCTGCACA GCAGCGGCCG CACGACCTC AAGTACTCCT ACCGCTTCTT | 255 |
| CONSENSUS | GACCTTCACA GTAGCGGCCG CACGACCTC MGTACTCTT ACCGCTTTGT | 550 |
| MOUSE DELTA DNA | GTGTGACGAG CACTACTACG GAGAAGGTG CTCGTCTTC TGCCGACCTC | 593 |
| HUMAN DELTA | GTGTGACGAA CACTACTACG GAGAGGGCTG CTCGTTTTC TGCCGTCCCC | 305 |
| CONSENSUS | GTGTGACGAR CACTACTACG GAGAGGYTG CTCGTCTTC TGCCGACCYC | 600 |
| MOUSE DELTA DNA | GGGATGACCC CTTTGGCCAC TTCACCTGG GGGACACAGG GGAGAAGATG | 643 |
| HUMAN DELTA | GGGACGATCC CTTCGGCCAC TTCACCTGT GGGAGCCTGG GGAGAAAGTG | 355 |
| CONSENSUS | GGGATGAYGC CTTTGGCCAC TTCACCTGY GGGAGMGGG GGAGAAARTG | 650 |
| MOUSE DELTA DNA | TGCAACCTG GCTGGAAAGG CCAGTACTGC GCTGACCCAA TCTGCTGCC | 693 |
| HUMAN DELTA | TGCAACCTG GCTGGAAAGG GGCCTACTGC ACAGAGCCGA TCTGCTGCC | 405 |
| CONSENSUS | TGCAACCTG GCTGGAAAGG SCMTACTGC ACAGASCORA TCTGYCTGCC | 700 |
| MOUSE DELTA DNA | AGCGTGTGAT GACCAACATG GATACTGTGA CAAACCAGGG GAGTGCAACT | 743 |
| HUMAN DELTA | TGGATGTGAT GACCAACATG GATTTTGTGA CAAACCAGGG GAGTGCAACT | 455 |
| CONSENSUS | WGGTGTGAT GACCAACATG GATWTTGTGA CAAACCAGGG GARTGCAACT | 750 |
| MOUSE DELTA DNA | GCAGAGTTGG CTGGCAGGGC CGTACTGGG ATGAGTGAAT CCGATATCCA | 793 |
| HUMAN DELTA | GCAGAGTGG CTGGCAGGGC CGTACTGTG ACAGTGTAT CCGTATCCA | 505 |
| CONSENSUS | GCAGAGTGG CTGGCAGGGC CGTACTGYS ATGAGTGMAT CCGMTATCCA | 800 |
| MOUSE DELTA DNA | GGTGTCTCC ATGGCACCTG CCAGCAACCC TGGCAGTGTA ACTGCCAGGA | 843 |
| HUMAN DELTA | GGTGTCTCC ATGGCACCTG CCAGCAGCCC TGGCAGTGA ACTGCCAGGA | 555 |
| CONSENSUS | GGTGTCTCC ATGGCACCTG CCAGCAACCC TGGCAGTGYA ACTGCCAGGA | 850 |
| MOUSE DELTA DNA | AGGCTGGGG GGCCTTTTCT GCAACCAGA CCTGAACCTAC TGTACTCACC | 893 |
| HUMAN DELTA | AGGNTGGGG GGCCTTTTCT GCAACCAGA CCTGAACCTAC TGCACACACC | 605 |
| CONSENSUS | AGGNTGGGG GGCCTTTTCT GCAACCAGA CCTGAACCTAC TGYACACACC | 900 |

FIG. 13B
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| | | |
|-----------------|---|------|
| MOUSE DELTA DNA | ATAAGCCGTC CAGGAATGGA GCCACCTGCA CCAACACGG GCCAGGGG-A | 941 |
| HUMAN DELTA | ATAAGCCGTC CAGGAATGGA GCCACCTGCA ACAAACACGG GCCAGGGGGA | 655 |
| CONSENSUS | ATAAGCCGTC CAGGAATGGA GCCACCTGCA ACAAACACGG GCCAGGGGGA | 950 |
| MOUSE DELTA DNA | GCTACACATG TTCCT-GCC-GACCTGGGT ATTACA-GGTG CCAACTGTG- | 986 |
| HUMAN DELTA | GCTACACTTG GTCCTTGGCC GGCTGGGGT ACAAAGGGTG CCACCTGGGA | 705 |
| CONSENSUS | GCTACACNTG KTCCTTGGCC GGCTGGGGT ACAAAGGGTG CCACCTGTGA | 1000 |
| MOUSE DELTA DNA | AGCT-GGAA GTAGATGAG-TG-TGCTCCT AGCCCTT-GC AAGAACGGAG | 1031 |
| HUMAN DELTA | AGCTTGGGGA TTGGACGAGT TGTGTACCCC AGCCCTTGGT AAGAACGGAG | 755 |
| CONSENSUS | AGCTTGGGGA KTRGAYGAGT TGTGTMYCCY AGCCCTTGGY AAGAACGGAG | 1050 |
| MOUSE DELTA DNA | GGAGCTGCAC GGACCTT-G AGCAGACGTT CTCCTTGACC TGCCCTCCCG | 1079 |
| HUMAN DELTA | GGAGCTTGAC GGATCTTCGG AGAACAGCTA CTCCTGTACC TGCCCAACCG | 805 |
| CONSENSUS | SGAGCTKSAC GGATCTTCGG AGRACAGCTW CTCCTGTACC TGCCCAACCG | 1100 |
| MOUSE DELTA DNA | GCTTCTATGG CAAGGTCTGT GAGGTGAGG CCATGACCTG TGGAGATGGC | 1129 |
| HUMAN DELTA | GCTTCAACGG CAAATCTGT GAATTGAGTG CCATGACCTG TGGGACGGC | 855 |
| CONSENSUS | GCTTCTAYGG CAARRTCTGT GARYTGAGYG CCATGACCTG TGGGAYGGC | 1150 |
| MOUSE DELTA DNA | CCTTGCTTCA ATGGAGGACG ATGTTTCAGAT AACCTGACG GAGGCTACAC | 1179 |
| HUMAN DELTA | CCTTGCTTTA ACGGGGTGCG GTGCTCAGAC AGCCCGGATG GAGGCTACAG | 905 |
| CONSENSUS | CCTTGCTTYA AYGGGGGNGG RTGTTTCAGAY ARCCCGGAYG GAGGCTACAS | 1200 |
| MOUSE DELTA DNA | CTGCCATGCG CCCTTGGGCT TCTCTGGCTT CAACTGTGAG AAGAAGATGG | 1229 |
| HUMAN DELTA | CTGCCGCTGC CCCGTGGGCT ACTCCGGCTT CAACTGTGAG AAGAAATTTG | 955 |
| CONSENSUS | CTGCCORYTG CCCTTGGGCT TCTCTGGCTT CAACTGTGAG AAGAARATGG | 1250 |
| MOUSE DELTA DNA | ATCTCTGCGG CTCCTCCCTT TGTCTAAGG GTGCCAAGTG TGTGGACCTC | 1279 |
| HUMAN DELTA | ACTACTGCAG CTCCTCACCC TGTCTAATG GTGCCAAGTG TGTGGACCTC | 1005 |
| CONSENSUS | AYYCTGCRG CTCCTCCCY TGTCTAAYG GTGCCAAGTG TGTGGACCTC | 1300 |
| MOUSE DELTA DNA | GGCAATCTTT ACCTGTGCCG CTGCCAGGCT GGCTTCTCGG GGAGGTACTG | 1329 |
| HUMAN DELTA | GGTGAATGCT ACCTGTGCCG CTGCCAGGCC GGCTTCTCGG GGAGGCACTG | 1055 |
| CONSENSUS | GGYRATKCYT ACCTGTGCCG CTGCCAGGCY GGCTTCTGSS GGAGGYACTG | 1350 |
| MOUSE DELTA DNA | CGAGGACAAT GTGGATGACT GTCCCTCCTC CCCGTGTGCA AATGGGGGCA | 1379 |
| HUMAN DELTA | TGAGGACAAC GTGGAGGACT GCGCTCCTC CCCGTGGGCC AAGGGGGGCA | 1105 |
| CONSENSUS | YGAGGACAAY GTGGAYGACY GYGCCTCCTC CCCGTGYGCM AAYGGGGGCA | 1400 |

FIG.13C

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|-----------------|------------|------------|--------------|------------|------------|------|
| MOUSE DELTA DNA | CCTGCCGGGA | CAGTGTGAAC | GACTTCTCCT | GTACCTGCCC | ACCTGGCTAC | 1429 |
| HUMAN DELTA | CCTGCCGGGA | TGCGTGAAC | GACTTCTCCT | GCACCTGCCC | GCCTGGCTAC | 1155 |
| CONSENSUS | CCTGCCGGGA | YRCYGTGAAC | GACTTGTCTCCT | GYACCTGCCC | RCCYGGCTAC | 1450 |
| MOUSE DELTA DNA | ACGGGCAAGA | ACTGCAGGCG | CCCTGTGAGC | AGGTGTGAGC | ATGCACCCTG | 1479 |
| HUMAN DELTA | ACGGGCAGGA | ACTGCAGTGC | CCCGGCCAGC | AGGTGCGAGC | ACGCACCCTG | 1205 |
| CONSENSUS | ACGGGCARGA | ACTGCAGYGC | CCCYGTGAGC | AGGTGYGAGC | AYGCACCCTG | 1500 |
| MOUSE DELTA DNA | CCATAATGGG | GCCACCTGCC | ACCAGAGGGG | CCACCGCTAC | ATGTGTGAGT | 1529 |
| HUMAN DELTA | CCATAATGGG | GCCACCTGCC | ACCAGAGGGG | CCACCGCTAT | TGTGTGAGT | 1255 |
| CONSENSUS | CCATAATGGG | GCCACCTGCC | ACCAGAGGGG | CCACCGCTAY | WTGTGYGAGT | 1550 |
| MOUSE DELTA DNA | GCGCCGAGG | CTATGGCGG | CCCAACTGCC | AGTTTCTGCT | CCCTGAGCC | 1578 |
| HUMAN DELTA | GTGCGGAAG | CTACGGGGT | CCCAACTGCC | ANTTCTGCT | CCCGAAACT | 1305 |
| CONSENSUS | GYGCCRRRG | CTAYGGGGY | CCCAACTGCC | ANTTYCTGCT | CCCYGAARY | 1600 |
| MOUSE DELTA DNA | -ACCACAGG | GCCCATGGTG | GTGGACCTC | AGTGAGAGC | ATATGGAGA | 1625 |
| HUMAN DELTA | GCCCCCGG | CCCCACGGTG | GTGGAACTC | CCCTAAAAA | ACCTAAAAG | 1355 |
| CONSENSUS | GMCCMCMGG | SCCCAYGGTG | GTGGAAMCTC | MSYKARARM | AYMTARRAGR | 1650 |
| MOUSE DELTA DNA | GCCAGGGCG | GCCCTTCCCC | TGGTGGGCG | TGTGTGCCCG | GGTGGTCTT | 1675 |
| HUMAN DELTA | GCCGGGGCG | GCCCATCCCC | TGGTGGACG | TGTGTGCCCG | GGTCATCTT | 1405 |
| CONSENSUS | GCCGGGGCG | GCCCTTCCCC | TGGTGGMCG | TGTGTGCCCG | GGTSRTCTT | 1700 |
| MOUSE DELTA DNA | GTCTCTTGC | TGCTGCTGGG | CTGTGCTGCT | GTGGTGGTCT | CGCTCCGGCT | 1725 |
| HUMAN DELTA | GTCTCATGC | TGCTGCTGGG | CTGTGCCGCT | GTGGTGGTCT | CGCTCCGGCT | 1455 |
| CONSENSUS | GTCTCTMTGC | TGCTGCTGGG | CTGTGCTGCT | GTGGTGGTCT | CGCTCCGGCT | 1750 |
| MOUSE DELTA DNA | GAGCTACAG | AACACCAAGC | GTCCATCTGA | ACCCTGTGGG | GGAGAGACAG | 1775 |
| HUMAN DELTA | GAGCTGCAG | AAGCACCGGC | CCCCATCGA | CCCCTGNCGG | GGGAGACGG | 1505 |
| CONSENSUS | GAGCTTCAG | AARCACCGGC | GYCCASCTGA | MCCCTGNSGG | GGGAGAACG | 1800 |
| MOUSE DELTA DNA | AAACCATGAA | CAACCTAGCC | AATTGCCAGC | GGGAGAAGGA | CGTTTCTGTT | 1825 |
| HUMAN DELTA | AGACCATGAA | CAACCTGGNC | AATTGCCAGC | GTGAGAAGGA | CATTCTAGTC | 1555 |
| CONSENSUS | AAACCATGAA | CAACCTAGNC | AAYTGCCAGC | GYGAGAAGGA | CRITYTNGTY | 1850 |

FIG.13D

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| | | | | | | |
|-----------------|--------------|------------|------------|------------|------------|------|
| MOUSE DELTA DNA | AGCATCATTC | GGGCTACCA | GATCAAGAAC | ACCAACAAGA | AGGCGGACTT | 1875 |
| HUMAN DELTA | AGCATCATTC | GGGNACGCA | GATCAAGAAC | ACCAACAAGA | AGGCGGACTT | 1605 |
| CONSENSUS | AGCATCATTC | GGGNYACCA | GATCAAGAAC | ACCAACAAGA | AGGCGGACTT | 1900 |
| MOUSE DELTA DNA | TCACGGGGAC | CATCGAGCCA | AGAAGAGCAG | CTTTAAGGTC | CGATACCCCA | 1925 |
| HUMAN DELTA | CCACGGGGAC | CACAGNGCCG | AGAAGAATGG | CTTCAAGGCC | CGCTACCCAG | 1655 |
| CONSENSUS | YCACGGGGAC | CAYRNGCCR | ASAAGARYRG | CTTMAAGGYC | CGMTACCCMR | 1950 |
| MOUSE DELTA DNA | CTGTGGACTA | TAACCTCGTT | CGAGACCTCA | AGGGAGATGA | AGCCACGGTC | 1975 |
| HUMAN DELTA | NGGTGGACTA | TAACCTCGTG | CAGGACCTCA | AGGGTGAAGA | CACCGCGTC | 1705 |
| CONSENSUS | NKGTGGACTA | TAACCTCGTK | CRRGACCTCA | AGGGAGATGA | MRCRCGSGTC | 2000 |
| MOUSE DELTA DNA | AGGGATACAC | ACAGCAACG | TGACACCAAG | TGCCAGTCAC | AGAGCTCTGC | 2025 |
| HUMAN DELTA | AGGGACGGCC | ACAGCAACG | TGACACCAAG | TGNCAGCCCC | AGGGCTCTTC | 1755 |
| CONSENSUS | AGGGAYRCRC | ACAGCAACG | TGACACCAAG | TGNCAGYCMC | AGRGCTCYKC | 2050 |
| MOUSE DELTA DNA | AGGAGAAGAG | AA—GATCG | CC—CCAACA | CTTTA—GGGT | GG—GG—AGAT | 2067 |
| HUMAN DELTA | AGGGGAGGAG | AAGGGGACCC | CGACCCACA | CTCAGGGGGT | GGAGGAAGCA | 1805 |
| CONSENSUS | AGGRGARGAG | AAGGGGAYDS | CGACCMACA | CTTACGGGGT | GGAGGAAGMW | 2100 |
| MOUSE DELTA DNA | TCTTGACAGA | AAAAGGCCAG | AGTCT—GTC | TACTGTAC—T | TCAAAGGAC— | 2113 |
| HUMAN DELTA | TCTTGAAAGA | AAAAGGCCGG | ACTTCGGGCT | TCTTCAACTT | TCAAAGACA | 1855 |
| CONSENSUS | TCYTGAAGAGA | AAAAGGCCRG | ASTYYGGYY | TRYTONACTT | TCAAARGACA | 2150 |
| MOUSE DELTA DNA | —ACCAAGTAC | CAGTCGGTGT | ATGTTGTGTC | TGCAGAA—A | AGGATGAGTG | 2160 |
| HUMAN DELTA | ANCAANGTAC | AAGTCGGTGT | NGTCAATTTT | CGNAGGAGGA | AGGNTGACTG | 1905 |
| CONSENSUS | ANCAANGTAC | MACTCGGTGT | NYGTYMTKTC | YGNAGRAGGA | AGGNTGASTG | 2200 |
| MOUSE DELTA DNA | TGTTATA—CC | GACTGAGGT— | GTAAGATGGA | AGCGATGTGG | CAAAATTCCT | 2208 |
| HUMAN DELTA | CGTCATAGGA | ANTTGAGGTN | GTAAGATGGA | AG—TT—TC | —ANNIT— | 1945 |
| CONSENSUS | YGTIYATAGM | RNYTGAGCTN | GTAARNITGN | AGCGATGTGG | CAANNITCCC | 2250 |
| MOUSE DELTA DNA | ATTCTCTCTA | AATAAAATTC | CAAGGATATA | GCCTCGATGA | ATGCTGCTGA | 2258 |
| HUMAN DELTA | —GCA AAGNNN— | TC CCGGAT— | —TCCGNT— | —TTC— | — | 1972 |
| CONSENSUS | ATTCTCKSA | AAKNNNATTC | CMGGATATA | GCYCCGNTGA | ATGCTKCTGA | 2300 |

FIG. 13E
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| | | | | | | |
|-----------------|------------|-------------|------------|------------|-------------|------|
| MOUSE DELTA DNA | GAGAGGAAGG | GAGAGGAAAC | CCAGGGACTG | CTGCTGAGAA | CCAGGTTTCAG | 2308 |
| HUMAN DELTA | ----- | AAA | ----- | GTTTTT | ----- | 1981 |
| CONSENSUS | GAGAGGAAGG | GAGAGGAAAC | CCAGGGACTG | YTKYTCAGAA | CCAGGTTTCAG | 2350 |
| MOUSE DELTA DNA | GCGAAGCTGG | TTCTCTCAGA | GTTAGCAGAG | GCGCCCGACA | CTGCCAGCCT | 2358 |
| HUMAN DELTA | ----- | ----- | ----- | ----- | ----- | 1981 |
| CONSENSUS | GCGAAGCTGG | TTCTCTCAGA | GTTAGCAGAG | GCGCCCGACA | CTGCCAGCCT | 2400 |
| MOUSE DELTA DNA | AGGCTTTGGC | TGCCGCTGGA | CTGCCTGCTG | GTTGTTCCCA | TTGCACTATG | 2408 |
| HUMAN DELTA | ----- | ----- | ----- | ----- | ----- | 1981 |
| CONSENSUS | AGGCTTTGGC | TGCCGCTGGA | CTGCCTGCTG | GTTGTTCCCA | TTGCACTATG | 2450 |
| MOUSE DELTA DNA | GACAGTTGCT | TTGAAGAGTA | TATATTTAAA | TGACGAGTG | ACTTGATTCA | 2458 |
| HUMAN DELTA | ----- | ----- | ----- | ----- | ----- | 1981 |
| CONSENSUS | GACAGTTGCT | TTGAAGAGTA | TATATTTAAA | TGACGAGTG | ACTTGATTCA | 2500 |
| MOUSE DELTA DNA | TATAGGAAGC | ACGCACTGCC | CACACGTCTA | TCTTGGATTA | CTATGAGCCA | 2508 |
| HUMAN DELTA | ----- | ----- | ----- | ----- | ----- | 1981 |
| CONSENSUS | TATAGGAAGC | ACGCACTGCC | CACACGTCTA | TCTTGGATTA | CTATGAGCCA | 2550 |
| MOUSE DELTA DNA | GTCTTTCCTT | GAAGTAGAAA | CACAACTGCC | TTTATTGTCC | TTTTTGATAC | 2558 |
| HUMAN DELTA | ----- | ----- | ----- | ----- | ----- | 1981 |
| CONSENSUS | GTCTTTCCTT | GAAGTAGAAA | CACAACTGCC | TTTATTGTCC | TTTTTGATAC | 2600 |
| MOUSE DELTA DNA | TGAGATGTGT | TTTTTTTTTT | CCTAGACGGG | AAAAAGAAAA | CGTGTGTTAT | 2608 |
| HUMAN DELTA | ----- | ----- | ----- | ----- | ----- | 1981 |
| CONSENSUS | TGAGATGTGT | TTTTTTTTTT | CCTAGACGGG | AAAAAGAAAA | CGTGTGTTAT | 2650 |
| MOUSE DELTA DNA | TTTTTGGGA | TTTGTA AAAA | TATTTTTCAT | GATATCTGTA | AAGCTTGAGT | 2658 |
| HUMAN DELTA | ----- | ----- | ----- | ----- | ----- | 1981 |
| CONSENSUS | TTTTTGGGA | TTTGTA AAAA | TATTTTTCAT | GATATCTGTA | AAGCTTGAGT | 2700 |
| MOUSE DELTA DNA | ATTTTGTGAC | GTTTCATTTT | TTATAATTTA | AATTTTGGTA | AATATGTACA | 2708 |
| HUMAN DELTA | ----- | ----- | ----- | ----- | ----- | 1981 |
| CONSENSUS | ATTTTGTGAC | GTTTCATTTT | TTATAATTTA | AATTTTGGTA | AATATGTACA | 2750 |

FIG.13F

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| | | |
|-----------------|---|------|
| MOUSE DELTA DNA | AAGGCACTTC GGGTCTATGT GACTATATTT TTTGTATAT AAATGTATTT | 2758 |
| HUMAN DELTA | ----- | 1981 |
| CONSENSUS | AAGGCACTTC GGGTCTATGT GACTATATTT TTTGTATAT AAATGTATTT | 2800 |
| MOUSE DELTA DNA | ATGGAATATT GTGCAAATGT TATTGAGTT TTTTACTGTT TTGTTAATGA | 2808 |
| HUMAN DELTA | ----- | 1981 |
| CONSENSUS | ATGGAATATT GTGCAAATGT TATTGAGTT TTTTACTGTT TTGTTAATGA | 2850 |
| MOUSE DELTA DNA | AGAAATTCAT TTAAAAATA TTTTCCAAA ATAAATATAA TGAAC TACA | 2857 |
| HUMAN DELTA | ----- | 1981 |
| CONSENSUS | AGAAATTCAT TTAAAAATA TTTTCCAAA ATAAATATAA TGAAC TACA | 2899 |

FIG.13G

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| | |
|-----------------------------------|-----|
| GFTWPGTFSLIIEALHTDSPD> | 21 |
| <u>DLATENPERLISRLATQRHL></u> | 41 |
| <u>TVGEEWSQDLHSSGRIDLKY></u> | 61 |
| <u>SYRFVCDEHYYGEGCSVFCR></u> | 81 |
| PRDDAFGHFTCGERGEKVCN> | 101 |
| <u>PGWKGPYCTEPICLPGCDEQ></u> | 121 |
| <u>HGFCDKPGECKCRVGWOGRY></u> | 141 |
| <u>CDECIRYPGCLHGTCQOPWQ></u> | 161 |
| <u>CNCOEGWGGLEFCNODLNYCT></u> | 181 |
| HHKPCKNGAIC* <u>TNTGQG*</u> | 198 |
| SYT* <u>PSP*KNGGSLTDL*</u> | 213 |
| ENSYSCTCPPGEYGKICELSAM> | 235 |
| <u>TCADGPCEFGGRCSDSPDGG></u> | 255 |
| <u>YSCRCPVGYSGFENCEKKIDY></u> | 275 |
| <u>CSSSPCSNGAKCVDLGDAYL></u> | 295 |
| <u>CRCOAGFSGRHCDDNVDDCA></u> | 315 |
| <u>SSPCANGGTCRDGVNDFSCT></u> | 335 |
| <u>CPPGYTGRNCSAPASRCEHA></u> | 355 |
| <u>PCHNGATCHERGHRY*CECA></u> | 374 |
| <u>RSYGGPNC*FLLPE*PPGP*></u> | 391 |
| <u>VV*LLLGCAAVVVCVRLRLQKH></u> | 412 |
| <u>RPPADP*RGETETMNNL*></u> | 428 |

FIG. 14A
SUBSTITUTE SHEET (RULE 26)

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| | |
|---|-----|
| <u>NCOREKDISVSIIG</u> * <u>TOIKNTN</u> > | 449 |
| <u>KKADFHGDH</u> * <u>ADKNGFKARYP</u> * | 469 |
| <u>VDYNLVQDLKGDDTAVRDAHSKRDTK</u> * | 494 |
| <u>OPOGSSGEEKGTP</u> * PTLR * GG * | 514 |
| <u>I</u> * <u>RKRP</u> * S * ST * SKD * T * | 526 |
| CVI * EV * | 531 |

FIG. 14B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11178

| | | | | | | | | | | | | | | |
|---|--|--|--|---|--|--|--|--|---|---|--|--|--|--|
| A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : C07H 17/00; C07K 14/00; C12P 21/06; C12N 5/00, 15/00
US CL : 536/23.1; 530/350; 435/69.1, 320.1, 240.1; 514/12, 44
According to International Patent Classification (IPC) or to both national classification and IPC | | | | | | | | | | | | | | |
| B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/23.1; 530/350; 435/69.1, 320.1, 240.1; 514/12, 44
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
None
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, Dialog | | | | | | | | | | | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | | | | | | | | | | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | | | | | | | | | | | | |
| Y | KOPCZYNSKI et al. Delta, a Drosophila neurogenic gene, is transcriptionally complex and encodes a protein related to blood coagulation factors and epidermal growth factor of vertebrates. Genes & Development. December 1988, Vol. 2, pages 1723-1735, see entire document. | 12, 13, 39, 56 | | | | | | | | | | | | |
| Y | VASSIN et al. The neurogenic gene Delta of Drosophila melangaster is expressed in neurogenic territories and encodes a putative transmembrane protein with EGF-like repeats. EMBO Journal, 1987, Vol. 6, No. 11, pages 3431-3440, see entire document. | 12, 13, 39, 56 | | | | | | | | | | | | |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex. | | | | | | | | | | | | | | |
| <table border="0"><tr><td>* Special categories of cited documents:</td><td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td></tr><tr><td>"A" document defining the general state of the art which is not considered to be of particular relevance</td><td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td></tr><tr><td>"B" earlier document published on or after the international filing date</td><td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td></tr><tr><td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td><td>"&" document member of the same patent family</td></tr><tr><td>"O" document referring to an oral disclosure, use, exhibition or other means</td><td></td></tr><tr><td>"P" document published prior to the international filing date but later than the priority date claimed</td><td></td></tr></table> | | | * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | "B" earlier document published on or after the international filing date | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" document member of the same patent family | "O" document referring to an oral disclosure, use, exhibition or other means | | "P" document published prior to the international filing date but later than the priority date claimed | |
| * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention | | | | | | | | | | | | | |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone | | | | | | | | | | | | | |
| "B" earlier document published on or after the international filing date | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art | | | | | | | | | | | | | |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" document member of the same patent family | | | | | | | | | | | | | |
| "O" document referring to an oral disclosure, use, exhibition or other means | | | | | | | | | | | | | | |
| "P" document published prior to the international filing date but later than the priority date claimed | | | | | | | | | | | | | | |
| Date of the actual completion of the international search
26 SEPTEMBER 1996 | | Date of mailing of the international search report
31 OCT 1996 | | | | | | | | | | | | |
| Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230 | | Authorized officer <i>K. Cochrane Carlson</i>
Karen Cochrane Carlson, Ph.D.
Telephone No. (703) 308-0196 | | | | | | | | | | | | |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11178

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/11178

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

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Group I, claim(s) 1-28, 49-56, 75-81, 84-89, 93, 94, drawn to vertebrate Delta protein.

Group II, claim(s) 29-32, 60, and 61, drawn to antibodies against vertebrate Delta.

Group III, claim(s) 33-48, 57-59, 70, 71, 82, 83, 90-92, 95, 96, 98, drawn to DNA encoding vertebrate Delta.

Group IV, claim(s) 62-65 and 69, drawn to a methods for the treatment or prevention of a disease with vertebrate Delta.

Group V, claim(s) 66, 67, and 72, drawn to a method for the treatment or prevention of disease via gene therapy.

Group VI, claim(s) 68, drawn to a method for the treatment or prevention of disease with the antibody.

Group VII, claim(s) 73, drawn to a method for diagnosing disease via Notch:Delta binding.

Group VIII, claim(s) 74, drawn to method for diagnosing disease via Delta levels.

The inventions listed as Groups I-VIII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Delta.

Group I is independent of Group II, IV, or VII because while the protein is used to make the antibody or in the methods, the protein can be used in either the methods or in making the antibody. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group I and III are independent because while the Delta protein can be made recombinantly from the DNA, this protein can also be made recombinantly. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group I is independent of Groups V, VI, and VIII because the protein is not used in any of these methods. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group II is independent of Group III because while the DNA is used to make the protein necessary in making the antibody, these products are unrelated in composition and activity. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group II is independent of Groups IV, V, VII, and VIII because the antibody is not used in any of these methods. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group II is independent of Group VI because while the antibody is used in the method, it can also be used in isolating Delta. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group III is independent of Groups IV, VI, VII, and VIII because the DNA is not used in any of these methods. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Group III is independent of Group V because while the DNA is used in the method, it can also be used to make the Delta protein recombinantly. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

Groups IV-VIII are independent one from the other because each method uses different products and methods steps to achieve different end results. Therefore, these Groups do not relate to a single inventive concept under PCT Rule 13.1.

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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